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(54) Title: HAEMOPHILUS ADHESION PROTEINS

(57) Abstract

The invention relates to novel *Haemophilus* adhesion proteins, nucleic acids, and antibodies.

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## ***HAEMOPHILUS ADHESION PROTEINS***

The U.S. Government has certain rights in this invention pursuant to grant numbers AI-21707 and HD-29687 from National Institutes of Health.

### **FIELD OF THE INVENTION**

5      The invention relates to novel *Haemophilus* adhesion proteins, nucleic acids, and antibodies.

### **BACKGROUND OF THE INVENTION**

Most bacterial diseases begin with colonization of a particular mucosal surface (Beachey et al., 1981, J. Infect. Dis. 143:325-345). Successful colonization requires 10 that an organism overcome mechanical cleansing of the mucosal surface and evade the local immune response. The process of colonization is dependent upon specialized microbial factors that promote binding to host cells (Hultgren et al., 1993 Cell, 73:887-901). In some cases the colonizing organism will subsequently enter (invade) these cells and survive intracellularly (Falkow, 1991, Cell 65:1099-15 1102).

*Haemophilus influenzae* is a common commensal organism of the human respiratory tract (Kuklinska and Kilian, 1984, Eur. J. Clin. Microbiol. 3:249-252). It is the most

common cause of bacterial meningitis and a leading cause of other invasive (bacteraemic) diseases. In addition, this organism is responsible for a sizeable fraction of acute and chronic otitis media, sinusitis, bronchitis, and pneumonia.

5 *Haemophilus influenzae* is a human-specific organism that normally resides in the human nasopharynx and must colonize this site in order to avoid extinction. This microbe has a number of surface structures capable of promoting attachment to host cells (Guerina *et al.*, 1982, *J. Infect. Dis.* 146:564; Pichichero *et al.*, 1982, *Lancet* ii:960-962; St. Geme *et al.*, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:2875-2879).  
10 In addition, *H. influenzae* has acquired the capacity to enter and survive within these cells (Forsgren *et al.*, 1994, *Infect. Immun.* 62:673-679; St. Geme and Falkow, 1990, *Infect. Immun.* 58:4036-4044; St. Geme and Falkow, 1991, *Infect. Immun.* 59:1325-1333, *Infect. Immun.* 59:3366-3371). As a result, this bacterium is an important cause of both localized respiratory tract and systemic disease (Turk, 1984, *J. Med. Microbiol.* 18:1-16). Nonencapsulated, non-typable strains account for the majority of local disease (Turk, 1984, *supra*); in contrast, serotype b strains, which express a capsule composed of a polymer of ribose and ribitol-5-phosphate (PRP), are responsible for over 95% of cases of *H. influenzae* systemic disease (Turk, 1982, *Clinical importance of Haemophilus influenzae*, p. 3-9. In S.H. Sell and P.F. Wright (ed.), *Haemophilus influenzae* epidemiology, immunology, and prevention of disease. Elsevier/North-Holland Publishing Co., New York).  
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25 The initial step in the pathogenesis of disease due to *H. influenzae* involves colonization of the upper respiratory mucosa (Murphy *et al.*, 1987, *J. Infect. Dis.* 5:723-731). Colonization with a particular strain may persist for weeks to months, and most individuals remain asymptomatic throughout this period (Spinola *et al.*, 1986, *J. Infect. Dis.* 154:100-109). However, in certain circumstances colonization will be followed by contiguous spread within the respiratory tract, resulting in local disease in the middle ear, the sinuses, the conjunctiva, or the lungs. Alternatively,

on occasion bacteria will penetrate the nasopharyngeal epithelial barrier and enter the bloodstream.

*In vitro* observations and animal studies suggest that bacterial surface appendages called pili (or fimbriae) play an important role in *H. influenzae* colonization. In 5 1982 two groups reported a correlation between piliation and increased attachment to human oropharyngeal epithelial cells and erythrocytes (Guerina *et al.*, *supra*; Pichichero *et al.*, *supra*). Other investigators have demonstrated that anti-pilus antibodies block *in vitro* attachment by pilated *H. influenzae* (Forney *et al.*, 1992. *J. Infect. Dis.* 165:464-470; van Alphen *et al.*, 1988. *Infect. Immun.* 56:1800-1806).

10 Recently Weber *et al.* insertionally inactivated the pilus structural gene in an *H. influenzae* type b strain and thereby eliminated expression of pili; the resulting mutant exhibited a reduced capacity for colonization of year-old monkeys (Weber *et al.*, 1991. *Infect. Immun.* 59:4724-4728).

15 A number of reports suggest that nonpilus factors also facilitate *Haemophilus* colonization. Using the human nasopharyngeal organ culture model, Farley *et al.* (1986. *J. Infect. Dis.* 161:274-280) and Loeb *et al.* (1988. *Infect. Immun.* 49:484-489) noted that nonpiliated type b strains were capable of mucosal attachment. Read and coworkers made similar observations upon examining nontypable strains in a model that employs nasal turbinate tissue in organ culture (1991. *J. Infect. Dis.* 20 163:549-558). In the monkey colonization study by Weber *et al.* (1991. *supra*), nonpiliated organisms retained a capacity for colonization, though at reduced densities; moreover, among monkeys originally infected with the pilated strain, virtually all organisms recovered from the nasopharynx were nonpiliated. All of these observations are consistent with the finding that nasopharyngeal isolates from 25 children colonized with *H. influenzae* are frequently nonpiliated (Mason *et al.*, 1985. *Infect. Immun.* 49:98-103; Brinton *et al.*, 1989. *Pediatr. Infect. Dis. J.* 8:554-561).

Previous studies have shown that *H. influenzae* are capable of entering (invading) cultured human epithelial cells via a pili-independent mechanism (St. Geme and Falkow, 1990, *supra*; St. Geme and Falkow, 1991, *supra*). Although *H. influenzae* is not generally considered an intracellular parasite, a recent report suggests that these *in vitro* findings may have an *in vivo* correlate (Forsgren *et al.*, 1994, *supra*).  
5 Forsgren and coworkers examined adenoids from 10 children who had their adenoids removed because of longstanding secretory otitis media or adenoidal hypertrophy. In all 10 cases there were viable intracellular *H. influenzae*. Electron microscopy demonstrated that these organisms were concentrated in the reticular crypt epithelium and in macrophage-like cells in the subepithelial layer of tissue. One possibility is that bacterial entry into host cells provides a mechanism for evasion  
10 of the local immune response, thereby allowing persistence in the respiratory tract.

Thus, a vaccine for the therapeutic and prophylactic treatment of *Haemophilus* infection is desirable. Accordingly, it is an object of the present invention to provide for recombinant *Haemophilus* Adherence (HA) proteins and variants thereof, and to produce useful quantities of these HA proteins using recombinant DNA  
15 techniques.

It is a further object of the invention to provide recombinant nucleic acids encoding HA proteins, and expression vectors and host cells containing the nucleic acid  
20 encoding the HA protein.

An additional object of the invention is to provide monoclonal antibodies for the diagnosis of *Haemophilus* infection.

A further object of the invention is to provide methods for producing the HA proteins, and a vaccine comprising the HA proteins of the present invention.

Methods for the therapeutic and prophylactic treatment of *Haemophilus* infection are also provided.

#### SUMMARY OF THE INVENTION

In accordance with the foregoing objects, the present invention provides recombinant 5 HA proteins, and isolated or recombinant nucleic acids which encode the HA proteins of the present invention. Also provided are expression vectors which comprise DNA encoding a HA protein operably linked to transcriptional and translational regulatory DNA, and host cells which contain the expression vectors.

10 The invention provides also provides methods for producing HA proteins which comprises culturing a host cell transformed with an expression vector and causing expression of the nucleic acid encoding the HA protein to produce a recombinant HA protein.

15 The invention also includes vaccines for *Haemophilus influenzae* infection comprising an HA protein for prophylactic or therapeutic use in generating an immune response in a patient. Methods of treating or preventing *Haemophilus influenzae* infection comprise administering a vaccine.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, and 1C depict the nucleic acid sequence of HA1.

Figure 2 depicts the amino acid sequence of HA1.

20 Figures 3A, 3B, 3C, 3D, 3E, 3F and 3G depict the nucleic acid sequence and amino acid sequence of HA2.

Figure 4 shows the schematic alignment of HA1 and HA2. Regions of sequence similarity are indicated by shaded, striped, and open bars, corresponding to N-terminal domains, internal domains, and C-terminal domains, respectively. The solid circles represent a conserved Walker box ATP-binding motif (GINVSGKT).  
5 Numbers above the bars refer to amino acid residue positions in the full-length proteins. Numbers in parentheses below the HA2 bars represent percent similarity/percent identity between these domains and the corresponding HA1 domains. The regions of HA2 defined by amino acid residues 51 to 173, 609 to 846, and 1292 to 1475 show minimal similarity to amino acids 51 to 220 of HA1.

10 Figure 5 depicts the homology between the N-terminal amino acid sequences of HA1 and HA2. Single letter abbreviations are used for the amino acids. A line indicates identity between the residues, and two dots indicate conservative changes, i.e. similarity between residues.

Figure 6 depicts the restriction maps of phage 11-17 and plasmid pT7-7 subclones.

15 Figure 7 depicts the restriction map of pDC400 and derivatives. pDC400 contains a 9.1 kb insert from strain C54 cloned into pUC19. Vector sequences are represented by hatched boxes. Letters above the top horizontal line indicate restriction enzyme sites: Bg, BgIII; E, EcoRI; H, HindIII; P, PstI; S, SalI; Ss, SstI; X, XbaI. The heavy horizontal line with arrow represents the location of the *hsf* locus within pDC400 and the direction of transcription. The striated horizontal line represents the 3.3 kb intragenic fragment used as a probe for Southern analysis. The plasmid pDC602, 20 which is not shown, contains the same insert as pDC601, but in the opposite orientation.

25 Figure 8 shows the identification of plasmid-encoded proteins using the bacteriophage T7 expression system. Bacteria were radiolabelled with

trans-[<sup>35</sup>S]-label, and whole cell lysates were resolved on a 7.5% SDS-polyacrylamide gel. Proteins were visualized by autoradiography. Lane 1. *E. coli* BL21(DE3)/pT7-7 uninduced; lane 2, BL21(DE3)/pT7-7 induced; lane 3, BL21(DE3)/pDC602 uninduced; lane 4, BL21(DE3)/pDC602 induced; lane 5, 5 BL21(DE3)/pDC601 uninduced; lane 6, BL21(DE3)/pDC601 induced. The plasmids pDC602 and pDC601 are derivatives of pT7-7 that contain the 8.3 kb *Xba*I fragment from pDC400 in opposite orientations. The asterisk indicates the overexpressed protein in BL21(DE3)/pDC601.

10 Figure 9 depicts the southern analysis of chromosomal DNA from *H. influenzae* strains C54 and 11, probing with *HA2* versus *HA1*. DNA fragments were separated on a 0.7% agarose gel and transferred bidirectionally to nitrocellulose membranes prior to probing with either *HA1* or *HA2*. Lane 1. C54 chromosomal DNA digested with *Bgl*II; lane 2. C54 chromosomal DNA digested with *Cla*I; lane 3. C54 chromosomal DNA digested with *Pst*I; lane 4. 11 chromosomal DNA digested with 15 *Bgl*II; lane 5. 11 chromosomal DNA digested with *Cla*I; lane 6, 11 chromosomal DNA digested with *Xba*I. A. Hybridization with the 3.3 kb *Pst*I-*Bgl*II intragenic fragment of *HA2* from strain C54. B. Hybridization with the 1.6 kb *Sty*I-*Ssp*I intragenic fragment of *HA1* from strain 11.

20 Figure 10 depicts the comparison of cellular binding specificities of *E. coli* DH5 $\alpha$  harboring *HA2* versus *HA1*. Adherence was measured after incubating bacteria with eucaryotic cell monolayers for 30 minutes as described and was calculated by dividing the number of adherent colony forming units by the number of inoculated colony forming units (St. Geme et al., 1993). Values are the mean  $\pm$  SEM of measurements made in triplicate from representative experiments. The 25 plasmid pDC601 contains the *HA2* gene from *H. influenzae* strain C54, while pHMW8-5 contains the *HA1* gene from nontypable *H. influenzae* strain 11. Both pDC601 and pHMW8-5 were prepared using pT7-7 as the cloning vector.

Figure 11 depicts the comparison of the N-terminal extremities of HA2, HMW1, HMW2, AIDA-I, Tsh, and SepA. The N-terminal sequence of HA2 is aligned with those of HA1 (Barenkamp, S.J., and J.W. St. Geme, III. Identification of a second family of high molecular weight adhesion proteins expressed by nontypable *Haemophilus influenzae*. *Mol. Microbiol.*, in press.), HMW1 and HMW2 (Barenkamp, S.J., and E. Leininger. 1992. Cloning, expression, and DNA sequence analysis of genes encoding nontypeable *Haemophilus influenzae* high molecular weight surface-exposed proteins related to filamentous hemagglutinin of *Bordetella pertussis*. *Infect. Immun.* **60**:1302-1313.). AIDA-I (Benz, I., and M.A. Schmidt. 1992. AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic *Escherichia coli* strain 2787 (O126:H27), is synthesized via a precursor molecule. *Mol. Microbiol.* **6**:1539-1546.). Tsh (Provence, D. and R. Curtiss III. 1994. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. *Infect. Immun.* **62**:1369-1380.). and Sep A (Benjelloun-Touimi, Z., P.J. Sansonetti, and C. Parsot. 1995. SepA, the major extracellular protein of *Shigella flexneri*: autonomous secretion and involvement in tissue invasion. *Mol. Microbiol.* **17**:123-135.). A consensus sequence is shown on the lower line.

Figure 12 depicts the southern analysis of chromosomal DNA from epidemiologically distinct strains of *H. influenzae* type b. Chromosomal DNA was digested with *Bgl*II, separated on a 0.7% agarose gel, transferred to nitrocellulose, and probed with the 3.3 kb *Pst*I-*Bgl*II intragenic fragment of *hsf* from strain C54. Lane 1, strain C54; lane 2, strain 1081; lane 3, strain 1065; lane 4, strain 1058; lane 5, strain 1060; lane 6, strain 1053; lane 7, strain 1063; lane 8, strain 1069; lane 9, strain 1070; lane 10, strain 1076; lane 11, strain 1084.

Figure 13 depicts the southern analysis of chromosomal DNA from non-type b encapsulated strains of *H. influenzae*. Chromosomal DNA was digested with *Bgl*II.

separated on a 0.7% agarose gel, transferred to nitrocellulose, and probed with the 3.3 kb *PstI-BglII* intragenic fragment of *hsf* from strain C54. Lane 1, SM4 (type a); lane 2, SM72 (type c); lane 3, SM6 (type d); lane 4, Rd (type d); lane 5, SM7 (type e); lane 6, 142 (type e); lane 7, 327 (type e); lane 8, 351 (type e); lane 9, 134 (type f); lane 10, 219 (type f); lane 11, 346 (type f); lane 12, 503 (type f).

Figures 14A and 14B are the nucleic acid sequence of HA3.

Figure 15 is the amino acid sequence of HA3.

Figures 16A and 16B depict the homology between the amino acid sequences of HA1 and HA3. Single letter abbreviations are used for the amino acids. A line indicates identity between the residues, and two dots indicate conservative changes, i.e. similarity between residues.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel *Haemophilus* Adhesion (HA) proteins. In a preferred embodiment, the HA proteins are from *Haemophilus* strains, and in the preferred embodiment, from *Haemophilus influenzae*. In particular, *H. influenzae* encapsulated type b strains are used to clone the HA proteins of the invention. However, using the techniques outlined below, HA proteins from other *Haemophilus influenzae* strains, or from other bacterial species such as *Neisseria* spp. or *Bordetella* spp. may also be obtained.

Three HA proteins, HA1, HA2 and HA3, are depicted in Figures 2, 3 and 15, respectively. HA2 is associated with the formation of surface fibrils, which are involved in adhesion to various host cells. HA1 has also been implicated in adhesion to a similar set of host cells. When the HA1 or HA2 nucleic acid is expressed in

a non-adherent strain of *E. coli* as described below, the *E. coli* acquire the ability to adhere to human host cells. It should be noted that in the literature, HA1 is referred to as hia (*H. influenza* adherence) and HA2 is referred to as hsf (*Haemophilus* surface fibrils).

5 A HA protein may be identified in several ways. A HA nucleic acid or HA protein is initially identified by substantial nucleic acid and/or amino acid sequence homology to the sequences shown in Figures 1, 2, 3, 14 or 15. Such homology can be based upon the overall nucleic acid or amino acid sequence or portions thereof.

10 As used herein, a protein is a "HA protein" if the overall homology of the protein sequence to the amino acid sequence shown in Figures 2 and/or Figure 3 and/or Figure 15 is preferably greater than about 45 to 50%, more preferably greater than about 65% and most preferably greater than 80%. In some embodiments the homology will be as high as about 90 to 95 or 98%. That is, a protein that has at least 50% homology (or greater) to one, two or all three of the amino acid sequences 15 of HA1, HA2 and HA3 is considered a HA protein. This homology will be determined using standard techniques known in the art, such as the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res.* 12:387-395 (1984) or the BLASTX program (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)). The alignment may include the introduction of gaps in the sequences to be aligned. As 20 noted below, in the comparison of proteins of different lengths, such as HA1 and HA3 with HA2, the homology is determined on the basis of the length of the shorter sequence.

25 In a preferred embodiment, a HA protein is defined as having significant homology to either the N-terminal region or the C-terminal region, or both, of the HA1, HA2 and HA3 proteins depicted in Figures 4, 5 and 15. The N-terminal region of about 50 amino acids is virtually identical as between HA1 and HA3 (98% homology).

and as between either HA1 or HA3 and HA2 is 74%. As shown in Figure 11, the first 24 amino acids of the N-terminus of HA1 and HA2 has limited homology to several other proteins, but this homology is 50% or less. Thus, a HA protein may be defined as having homology to the N-terminal region of at least about 60%, preferably at least about 70%, and most preferably at least about 80%, with homology as high as 90 or 95% especially preferred. Similarly, the C-terminal region of at least about 75, preferably 100 and most preferably 125 amino acid residues is also highly homologous and can be used to identify a HA protein. As shown in Figure 16, the homology between the C-terminal 120 or so amino acids of HA1 and HA3 is about 98%, and as between either HA1 or HA3 and HA2 is also about 98%. Thus homology at the C-terminus is a particularly useful way of identifying a HA protein. Accordingly, a HA protein can be defined as having homology to the C-terminal region of at least about 60%, preferably at least about 70%, and most preferably at least about 80%, with homology as high as 90 or 95% especially preferred. In a preferred embodiment, the HA protein has homology to both the N- and C-terminal regions.

In addition, a HA protein may be identified as containing at least one stretch of amino acid homology found at least in the HA1 and HA2 proteins as depicted in Figure 4. HA2 contains three separate stretchs of amino acids (174 to 608, 847 to 1291, and 1476 to 1914, respectively) that shows significant homology to the region of HA1 defined by amino acids 221 to 658.

The HA proteins of the present invention have limited homology to the high molecular weight protein-1 (HMW1) of *H. influenzae*, as well as the AIDA-I adhesin of *E. coli*. For the HMW1 protein, this homology is greatest between residues 60-540 of the HA1 protein and residues 1100 to about 1550 of HMW1, with 20% homology in this overlap region. For the AIDA-I protein, there is a roughly 50%

homology between the first 30 amino acids of AIDA-I and HA1, and the overall homology between the proteins is roughly 22%.

In addition, the HA1, HA2 and HA3 proteins of the present invention have homology to each other, as shown in Figures 4, 5 and 16. As between HA1 and HA2, the homology is 81% similarity and 72% identity overall. HA3 and HA1 are 51% identical and 65% similar. Thus, for the purposes of the invention, HA1, HA2 and HA3 are all HA proteins.

An "HA1" protein is defined by substantial homology to the sequence shown in Figure 2. This homology is preferably greater than about 60%, more preferably greater than about 70% and most preferably greater than 80%. In preferred embodiments the homology will be as high as about 90 to 95 or 98%. Similarly, an "HA2" protein may be defined by the same substantial homology to the sequence shown in Figure 3, and a "HA3" protein is defined with reference to Figure 15, as defined above.

In addition, for sequences which contain either more or fewer amino acids than the proteins shown in Figures 2, 3 and 15, it is understood that the percentage of homology will be determined based on the number of homologous amino acids in relation to the total number of amino acids. Thus, for example, homology of sequences shorter than that shown in Figures 2, 3 and 15, as discussed below, will be determined using the number of amino acids in the shorter sequence.

HA proteins of the present invention may be shorter than the amino acid sequences shown in Figures 2, 3 and 15. Thus, in a preferred embodiment, included within the definition of HA proteins are portions or fragments of the sequence shown in Figures 2, 3 and 15. Generally, the HA protein fragments may range in size from about 7 amino acids to about 800 amino acids, with from about 15 to about 700

amino acids being preferred, and from about 100 to about 650 amino acids also preferred. Particularly preferred fragments are sequences unique to HA; these sequences have particular use in cloning HA proteins from other organisms, to generate antibodies specific to HA proteins, or for particular use as a vaccine.

5 Unique sequences are easily identified by those skilled in the art after examination of the HA protein sequence and comparison to other proteins; for example, by examination of the sequence alignment shown in Figures 5 and 16. Preferred unique sequences include the N-terminal region of the HA1, HA2 and HA3 sequences, comprising roughly 50 amino acids and the C-terminal 120 amino acids, depicted

10 in Figures 2, 3 and 15. HA protein fragments which are included within the definition of a HA protein include N- or C-terminal truncations and deletions which still allow the protein to be biologically active; for example, which still allow adherence, as described below. In addition, when the HA protein is to be used to generate antibodies, for example as a vaccine, the HA protein must share at least

15 one epitope or determinant with the sequences shown in Figures 2, 3 and 15. In a preferred embodiment, the epitope is unique to the HA protein; that is, antibodies generated to a unique epitope exhibit little or no cross-reactivity with other proteins. However, cross reactivity with other proteins does not preclude such epitopes or antibodies for immunogenic or diagnostic uses. By "epitope" or "determinant"

20 herein is meant a portion of a protein which will generate and/or bind an antibody. Thus, in most instances, antibodies made to a smaller HA protein will be able to bind to the full length protein.

25 In some embodiments, the fragment of the HA protein used to generate antibodies are small; thus, they may be used as haptens and coupled to protein carriers to generate antibodies, as is known in the art.

In addition, sequences longer than those shown in Figures 2, 3 and 15 are also included within the definition of HA proteins.

Preferably, the antibodies are generated to a portion of the HA protein which is exposed at the outer membrane, i.e. surface exposed. The amino-terminal portions of HA1, HA2 and HA3 are believed to be externally exposed proteins.

5 The HA proteins may also be identified as associated with bacterial adhesion. Thus, deletions of the HA proteins from the naturally occurring microorganism such as *Haemophilus* species results in a decrease or absence of binding ability. In some embodiments, the expression of the HA proteins in a non-adherent bacteria such as *E. coli* results in the ability of the organism to bind to cells.

10 In the case of the nucleic acid, the overall homology of the nucleic acid sequence is commensurate with amino acid homology but takes into account the degeneracy in the genetic code and codon bias of different organisms. Accordingly, the nucleic acid sequence homology may be either lower or higher than that of the protein sequence. Thus the homology of the nucleic acid sequence as compared to the nucleic acid sequences of Figures 1, 3 and 14 is preferably greater than about 40%, more preferably greater than about 60% and most preferably greater than 80%. 15 In some embodiments the homology will be as high as about 90 to 95 or 98%.

As outlined for the protein sequences, a preferred embodiment utilizes HA nucleic acids with substantial homology to the unique N-terminal and C-terminal regions of the HA1, HA2 and HA3 sequences.

20 In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to all or part of the nucleic acid sequences shown in Figures 1, 3 and 14 are considered HA protein genes. High stringency conditions include, but are not limited to, washes with 0.1XSSC at 65°C for 2 hours.

The HA proteins and nucleic acids of the present invention are preferably recombinant. As used herein, "nucleic acid" may refer to either DNA or RNA, or molecules which contain both deoxy- and ribonucleotides. The nucleic acids include genomic DNA, cDNA and oligonucleotides including sense and anti-sense nucleic acids. Specifically included within the definition of nucleic acid are anti-sense nucleic acids. An anti-sense nucleic acid will hybridize to the corresponding non-coding strand of the nucleic acid sequences shown in Figures 1, 3 and 14, but may contain ribonucleotides as well as deoxyribonucleotides. Generally, anti-sense nucleic acids function to prevent expression of mRNA, such that a HA protein is not made, or made at reduced levels. The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed in vitro by the manipulation of nucleic acid by endonucleases, in a form not normally found in nature. Thus an isolated HA protein gene, in a linear form, or an expression vector formed in vitro by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention; i.e. the HA nucleic acid is joined to other than the naturally occurring *Haemophilus* chromosome in which it is normally found. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell rather than in vitro manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention.

Similarly, a "recombinant protein" is a protein made using recombinant techniques, i.e. through the expression of a recombinant nucleic acid as depicted above. A recombinant protein is distinguished from naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated away from some or all of the proteins and compounds with which it is normally associated.

in its wild type host, or found in the absence of the host cells themselves. Thus, the protein may be partially or substantially purified. The definition includes the production of a HA protein from one organism in a different organism or host cell. Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of a inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Alternatively, the protein may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions. Furthermore, although not normally considered "recombinant", proteins or portions of proteins which are synthesized chemically, using the sequence information of Figures 2, 3 and 15, are considered recombinant herein as well.

Also included with the definition of HA protein are HA proteins from other organisms, which are cloned and expressed as outlined below.

In the case of anti-sense nucleic acids, an anti-sense nucleic acid is defined as one which will hybridize to all or part of the corresponding non-coding sequence of the sequences shown in Figures 1, 3 and 14. Generally, the hybridization conditions used for the determination of anti-sense hybridization will be high stringency conditions, such as 0.1XSSC at 65°C.

Once the HA protein nucleic acid is identified, it can be cloned and, if necessary, its constituent parts recombined to form the entire HA protein nucleic acid. Once isolated from its natural source, e.g., contained within a plasmid or other vector or excised therefrom as a linear nucleic acid segment, the recombinant HA protein nucleic acid can be further used as a probe to identify and isolate other HA protein nucleic acids. It can also be used as a "precursor" nucleic acid to make modified or variant HA protein nucleic acids and proteins.

Using the nucleic acids of the present invention which encode HA protein, a variety of expression vectors are made. The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the HA protein.

5 "Operably linked" in this context means that the transcriptional and translational regulatory DNA is positioned relative to the coding sequence of the HA protein in such a manner that transcription is initiated. Generally, this will mean that the promoter and transcriptional initiation or start sequences are positioned 5' to the HA protein coding region. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the HA protein: for example, transcriptional and translational regulatory nucleic acid sequences from 10 Bacillus will be used to express the HA protein in Bacillus. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

15

In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the 20 regulatory sequences include a promoter and transcriptional start and stop sequences.

Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

25 In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be

5        maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

10      In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

15      The HA proteins of the present invention are produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding a HA protein, under the appropriate conditions to induce or cause expression of the HA protein. The conditions appropriate for HA protein expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

20      Appropriate host cells include yeast, bacteria, archebacteria, fungi, and insect and animal cells, including mammalian cells. Of particular interest are Drosophila melanogaster cells, Saccharomyces cerevisiae and other yeasts, E. coli, Bacillus

subtilis, SF9 cells, C129 cells, 293 cells, Neurospora, BHK, CHO, COS, and HeLa cells, immortalized mammalian myeloid and lymphoid cell lines.

In a preferred embodiment, HA proteins are expressed in bacterial systems. Bacterial expression systems are well known in the art.

5      A suitable bacterial promoter is any nucleic acid sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of the coding sequence of HA protein into mRNA. A bacterial promoter has a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site. Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose and maltose, and sequences derived from biosynthetic enzymes such as tryptophan. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters are also useful: for example, the *tac* promoter is a hybrid of the *trp* and *lac* promoter sequences. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription.

10     

15     

20     In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable. In *E. coli*, the ribosome binding site is called the Shine-Delgarno (SD) sequence and includes an initiation codon and a sequence 3-9 nucleotides in length located 3 - 11 nucleotides upstream of the initiation codon.

25     The expression vector may also include a signal peptide sequence that provides for secretion of the HA protein in bacteria. The signal sequence typically encodes

a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell, as is well known in the art. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria).

5        The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan  
10      and leucine biosynthetic pathways.

These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for *Bacillus subtilis*, *E. coli*, *Streptococcus cremoris*, and *Streptococcus lividans*, among others.

15      The bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

20      In one embodiment, HA proteins are produced in insect cells. Expression vectors for the transformation of insect cells, and in particular, baculovirus-based expression vectors, are well known in the art. Briefly, baculovirus is a very large DNA virus which produces its coat protein at very high levels. Due to the size of the baculoviral genome, exogenous genes must be placed in the viral genome by recombination. Accordingly, the components of the expression system include: a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the HA protein; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment  
25      5

in the transfer vector (this allows for the homologous recombination of the heterologous gene into the baculovirus genome); and appropriate insect host cells and growth media.

5 Mammalian expression systems are also known in the art and are used in one embodiment. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence for HA protein into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, using a located 25-30 base pairs upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, typically located within 100 to 200 base pairs upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation. Of particular 10 use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, and herpes simplex virus promoter.

15

20 Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-translational cleavage and polyadenylation. Examples of transcription terminator and polyadenylation signals include those derived from SV40.

25 The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, is well known in the art, and will vary with the host cell used.

Techniques included extran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

5 In a preferred embodiment, HA protein is produced in yeast cells. Yeast expression systems are well known in the art, and include expression vectors for Saccharomyces cerevisiae, Candida albicans and C. maltosa, Hansenula polymorpha, Kluyveromyces fragilis and K. lactis, Pichia guillermondii and P. pastoris, Schizosaccharomyces pombe, and Yarrowia lipolytica. Preferred promoter sequences for expression in yeast include the inducible GAL1.10 promoter, the promoters from alcohol dehydrogenase, enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase, hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, pyruvate kinase, and the acid phosphatase gene. Yeast selectable markers include ADE2, HIS4, LEU2, TRP1, and ALG7, which confers resistance to tunicamycin; the G418 resistance gene, which confers resistance to G418; and the CUP1 gene, which allows yeast to grow in the presence of copper ions.

20 A recombinant HA protein may be expressed intracellularly or secreted. The HA protein may also be made as a fusion protein, using techniques well known in the art. Thus, for example, if the desired epitope is small, the HA protein may be fused to a carrier protein to form an immunogen. Alternatively, the HA protein may be made as a fusion protein to increase expression.

25 Also included within the definition of HA proteins of the present invention are amino acid sequence variants. These variants fall into one or more of three classes: substitutional, insertional or deletional variants. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the HA

protein, using cassette mutagenesis or other techniques well known in the art, to produce DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture as outlined above. However, variant HA protein fragments having up to about 100-150 residues may be prepared by *in vitro* synthesis using 5 established techniques. Amino acid sequence variants are characterized by the predetermined nature of the variation, a feature that sets them apart from naturally occurring allelic or interspecies variation of the HA protein amino acid sequence. The variants typically exhibit the same qualitative biological activity as the naturally occurring analogue, although variants can also be selected which have modified 10 characteristics as will be more fully outlined below.

While the site or region for introducing an amino acid sequence variation is predetermined, the mutation *per se* need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed HA protein 15 variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis. Screening of the mutants is done using assays of HA protein activities; for example, mutated HA genes are placed in HA deletion strains and tested for HA activity, as disclosed 20 herein. The creation of deletion strains, given a gene sequence, is known in the art. For example, nucleic acid encoding the variants may be expressed in an adhesion deficient strain, and the adhesion and infectivity of the variant *Haemophilus influenzae* evaluated. For example, as outlined below, the variants may be expressed in the *E. coli* DH5 $\alpha$  non-adherent strain, and the transformed 25 *E. coli* strain evaluated for adherence using Chang conjunctival cells.

Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about 1 to 20 amino acids, although considerably larger

insertions may be tolerated. Deletions range from about 1 to 30 residues, although in some cases deletions may be much larger, as for example when one of the domains of the HA protein is deleted.

Substitutions, deletions, insertions or any combination thereof may be used to arrive at a final derivative. Generally these changes are done on a few amino acids to minimize the alteration of the molecule. However, larger changes may be tolerated in certain circumstances.

When small alterations in the characteristics of the HA protein are desired, substitutions are generally made in accordance with the following chart:

### Chart I

<u>Original Residue</u>		<u>Exemplary Substitutions</u>
		Ser
10	Ala	Lys
	Arg	Gln. His
	Asn	Glu
15	Asp	Ser
	Cys	Asn
	Gln	Asp
	Glu	Pro
	Gly	Asn. Gln
20	His	Leu. Val
	Ile	Ile. Val
	Leu	Arg. Gln, Glu
	Lys	Leu. Ile
	Met	Met. Leu, Tyr
25	Phe	Thr
	Ser	Ser
	Thr	Tyr
	Trp	Trp. Phe
	Tyr	Ile. Leu
30	Val	

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in Chart I. For example, substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g. lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g. glycine.

15 The variants typically exhibit the same qualitative biological activity and will elicit the same immune response as the naturally-occurring analogue, although variants also are selected to modify the characteristics of the polypeptide as needed. Alternatively, the variant may be designed such that the biological activity of the HA protein is altered. For example, the Walker box ATP-binding motif may be altered or eliminated.

20 In a preferred embodiment, the HA protein is purified or isolated after expression. HA proteins may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing. For example, the HA protein may be purified using a standard anti-HA antibody column.

25

5 Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., *Protein Purification*, Springer-Verlag, NY (1982). The degree of purification necessary will vary depending on the use of the HA protein. In some instances no purification will be necessary.

10 Once expressed and purified if necessary, the HA proteins are useful in a number of applications.

15 For example, the HA proteins can be coupled, using standard technology, to affinity chromatography columns. These columns may then be used to purify antibodies from samples obtained from animals or patients exposed to the *Haemophilus influenzae* organism. The purified antibodies may then be used as outlined below.

20 Additionally, the HA proteins are useful to make antibodies to HA proteins. These antibodies find use in a number of applications. The antibodies are used to diagnose the presence of an *Haemophilus influenzae* infection in a sample or patient. In a preferred embodiment, the antibodies are used to detect the presence of nontypable *Haemophilus influenzae* (NTHI), although typable *H. influenzae* infections are also detected using the antibodies.

25 This diagnosis will be done using techniques well known in the art: for example, samples such as blood or tissue samples may be obtained from a patient and tested for reactivity with the antibodies, for example using standard techniques such as ELISA. In a preferred embodiment, monoclonal antibodies are generated to the HA protein, using techniques well known in the art. As outlined above, the antibodies may be generated to the full length HA protein, or a portion of the HA protein.

Antibodies generated to HA proteins may also be used in passive immunization treatments, as is known in the art.

5       Antibodies generated to unique sequences of HA proteins may also be used to screen expression libraries from other organisms to find, and subsequently clone, HA nucleic acids from other organisms.

10       In one embodiment, the antibodies may be directly or indirectly labelled. By "labelled" herein is meant a compound that has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the compound at any position. Thus, for example, the HA protein antibody may be labelled for detection, or a secondary antibody to the HA protein antibody may be created and labelled.

15       In one embodiment, the antibodies generated to the HA proteins of the present invention are used to purify or separate HA proteins or the *Haemophilus influenzae* organism from a sample. Thus for example, antibodies generated to HA proteins which will bind to the *Haemophilus influenzae* organism may be coupled, using standard technology, to affinity chromatography columns. These columns can be used to pull out the *Haemophilus* organism from environmental or tissue samples.

20       In a preferred embodiment, the HA proteins of the present invention are used as vaccines for the prophylactic or therapeutic treatment of a *Haemophilus influenzae* infection in a patient. By "vaccine" or "immunogenic compositions" herein is meant an antigen or compound which elicits an immune response in an animal or patient. The vaccine may be administered prophylactically, for example to a patient never previously exposed to the antigen, such that subsequent infection by the

5           *Haemophilus influenzae* organism is prevented. Alternatively, the vaccine may be administered therapeutically to a patient previously exposed or infected by the *Haemophilus influenzae* organism. While infection cannot be prevented, in this case an immune response is generated which allows the patient's immune system to more effectively combat the infection. Thus, for example, there may be a decrease or lessening of the symptoms associated with infection.

A "patient" for the purposes of the present invention includes both humans and other animals and organisms. Thus the methods are applicable to both human therapy and veterinary applications.

10           The administration of the HA protein as a vaccine is done in a variety of ways. Generally, the HA proteins can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby therapeutically effective amounts of the HA protein are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are well known in the art. Such compositions will contain an effective amount of the HA protein together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions for effective administration to the host. The composition may include salts, buffers, carrier proteins such as serum albumin, targeting molecules to localize the HA protein at the appropriate site or tissue within the organism, and other molecules. The composition may include adjuvants as well.

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25           In one embodiment, the vaccine is administered as a single dose; that is, one dose is adequate to induce a sufficient immune response to prophylactically or therapeutically treat a *Haemophilus influenzae* infection. In alternate embodiments, the vaccine is administered as several doses over a period of time, as a primary vaccination and "booster" vaccinations.

By "therapeutically effective amounts" herein is meant an amount of the HA protein which is sufficient to induce an immune response. This amount may be different depending on whether prophylactic or therapeutic treatment is desired. Generally, this ranges from about 0.001 mg to about 1 gm, with a preferred range of about 0.05 to about .5 gm. These amounts may be adjusted if adjuvants are used.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All references cited herein are specifically incorporated by reference.

#### EXAMPLE I

##### Cloning of HA1

Many protocols are substantially the same as those outlined in St. Geme et al., Mol. Microbiol. 15(1):77-85 (1995).

15 **Bacterial strains, plasmids, and phages.**

Nontypable *H. influenzae* strain 11 was the clinical isolate chosen as a prototypic HMW1/HMW2-non-expressing strain, although a variety of encapsulated typable strains can be used to clone the protein using the sequences of the figures. The organism was isolated in pure culture from the middle ear fluid of a child with acute otitis media. The strain was identified as *H. influenzae* by standard methods and was classified as nontypable by its failure to agglutinate with a panel of typing antisera for *H. influenzae* types a to f (Burroughs Wellcome Co., Research Triangle Park, N.C.) and failure to show lines of precipitation with these antisera in counterimmunoelectrophoresis assays. Strain 11 adheres efficiently to Chang

conjunctival cells *in vitro*, at levels comparable to those previously demonstrated for NTHI strains expressing HMW1/HMW2-like proteins (data not shown). Convalescent serum from the child infected with this strain demonstrated an antibody response directed predominantly against surface-exposed high molecular weight proteins with molecular weights greater than 100 kDa.

5

M13mp18 and M13mp19 were obtained from New England BioLabs, Inc. (Beverly, Mass.) pT7-7 was the kind gift of Stanley Tabor. This vector contains the T7 RNA polymerase promoter  $\phi$ 10, a ribosome-binding site, and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site.

10

#### Molecular cloning and plasmid subcloning.

15

The recombinant phage containing the *HA1* gene was isolated and characterized using methods similar to those described previously. In brief, chromosomal DNA from strain 11 was prepared and *Sau3A* partial restriction digests of the DNA were prepared and fractionated on 0.7% agarose gels. Fractions containing DNA fragments in the 9- to 20-kbp range were pooled, and a library was prepared by ligation into  $\lambda$ EMBL3 arms. Ligation mixtures were packaged *in vitro* with Gigapack (Stratagene) and plate-amplified in a P2 lysogen of *E. coli* LE392. Lambda plaque immunological screening was performed as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Ed. (1989), Cold Spring Harbor Press. For plasmid subcloning studies, DNA from recombinant phage was subcloned into the T7 expression plasmid pT7-7. Standard methods were used for manipulation of cloned DNA as described by Maniatis et al (supra).

20

Plasmid pHMW8-3 was generated by isolating an 11 kbp *Xba*1 fragment from purified DNA from recombinant phage clone 11-17 and ligating into *Xba*1 cut pT7-7. Plasmid pHMW8-4 was generated by isolating a 10 kbp *Bam*H1-C1a1 cut pT7-7.

25

Plasmid pHMW8-5 was generated by digesting plasmid pHMW8-3 DNA with *Clal*, isolating the larger fragment and religating. Plasmid pHMW8-6 was generated by digesting pHMW8-4 with *Spel*, which cuts at a unique site within the *HA1* gene, blunt-ending the resulting fragment, inserting a kanamycin resistance cassette into the *Spel* site. Plasmid pHMW8-7 was generated by digesting pHMW8-3 with *Nru1* and *HindIII*, isolating the fragment containing pT7-7, blunt-ending and religating. The plasmid restriction maps are shown in Figure 6.

**DNA sequence analysis.**

DNA sequence analysis was performed by the dideoxy method with the U.S. Biochemicals Sequenase kit as suggested by the manufacturer. [<sup>36</sup>S]dATP was purchased from New England Nuclear (Boston, Mass). Data were analyzed with Compugen software and the Genetics Computer Group program from the University of Wisconsin on a Digital VAX 8530 computer. Several 21-mer oligonucleotide primers were generated as necessary to complete the sequence.

**15 Adherence assays.**

Adherence assays were done with Chang epithelial cells [Wong-Kilbourne derivative, clone 1-5c-4 (human conjunctiva), ATCC CCL20.2)], which were seeded into wells of 24-well tissue culture plates, as described (St. Geme III et al., Infect. Immun. 58:4036 (1990)). Bacteria were inoculated into broth and allowed to grow to a density of approximately  $2 \times 10^9$  colony-forming units per ml. Approximately  $2 \times 10^7$  colony-forming units were inoculated onto epithelial cells monolayers, and plates were gently centrifuged at  $165 \times g$  for 5 min to facilitate contact between bacteria and the epithelial surface. After incubation for 30 min at  $37^\circ C$  in 5% CO<sub>2</sub>, monolayers were rinsed five times with phosphate buffered saline (PBS) to remove nonadherent organisms and were treated with trypsin-EDTA (0.05% trypsin/0.5%

EDTA) in PBS to release them from the plastic support. Well contents were agitated, and dilution were plated on solid medium to yield the number of adherent bacteria per monolayer. Percent adherence was calculated by dividing the number of adherent colony-forming units per monolayer by the number of inoculated colony-forming units.

5

Isolation and characterization of recombinant phage expressing the strain 11 high molecular weight adhesion protein.

10

15

The nontypable *Haemophilus influenzae* strain 11 chromosomal DNA library was screened immunologically with convalescent serum from the child infected with strain 11. Immunoreactive clones were screened by Western blot for expression of high molecular weight proteins with apparent molecular weights > 100 dDa and two different classes of recombinant clones were recovered. A single clone designated 11-17 was recovered which expressed the HA1 protein. The recombinant protein expressed by this clone had an apparent molecular weight of greater than 200 kDa.

Transformation into *E. coli*

Plasmids were introduced into DH5 $\alpha$  strain of *E. coli* (Maniatis, supra), which is a non-adherent strain, using electroporation (Dower et al., Nucl. Acids Res. 16:6127 (1988). The results are shown in Table 1.

**Table 1**

Strain	% Adherence
DH5 $\alpha$ (pHMW 8-4)	43.3 $\pm$ 5.0%
DH5 $\alpha$ (pHMW 8-5)	41.3 $\pm$ 3.3%
DH5 $\alpha$ (pHMW 8-6)	0.6 $\pm$ 0.3%
DH5 $\alpha$ (pHMW 8-7)	
DH5 $\alpha$ (pT7-7)	0.4 $\pm$ 0.1%

• Adherence was measured in a 30 minute assay and was calculated by dividing the number of adherent bacteria by the number of inoculated bacteria. Values are the mean  $\pm$  SEM of measurements made in triplicate from a representative experiment.

In addition, a monoclonal antibody made by standard procedures, directed against the strain 11 protein recognized proteins in 57 of 60 epidemiologically-unrelated NTHI. However, Southern analysis using the gene indicated that roughly only 25% of the tested strains actually hybridized to the gene (data not shown).

### EXAMPLE 2

## Cloning of HA?

In a recent study we examined a series of *H. influenza* type b isolates by transmission electron microscopy and visualized short, thin surface fibrils distinct from pili (St. Geme, J.W.III. and D. Cutter. 1995. Evidence that surface fibrils expressed by *Haemophilus influenzae* type b promote attachment to human epithelial cells. *Mol. Microbiol.* **15**:77-85.). In that study, the large genetic locus involved in the expression of these appendages was isolated.

## Bacterial strains and plasmids

*H. influenzae* strain C54 is a type b strain that has been described previously (Pichichero, M.E., P. Anderson, M. Loeb, and D.H. Smith. 1982. Do pili play a role in pathogenicity of *Haemophilus influenzae* type b? *Lancet.* **ii**:960-962.). Strain C54-Tn400.23 is a mutant that contains a mini-Tn10 *kan* element in the *hsf* locus and demonstrates minimal *in vitro* adherence (St. Geme, J.W.III, and D. Cutter. 5 1995. Evidence that surface fibrils expressed by *Haemophilus influenzae* type b promote attachment to human epithelial cells. *Mol. Microbiol.* **15**:77-85.). Strains 1053, 1058, 1060, 1063, 1065, 1069, 1070, 1076, 1081, and 1084 are *H. influenzae* type b isolates generously provided by J. Musser (Baylor University, Houston, 10 Texas) (Musser et al., 1990. Global genetic structure and molecular epidemiology of encapsulated *Haemophilus influenzae*. *Rev. Infect. Dis.* **12**:75-111.). *H. influenzae* strains SM4 (type a), SM6 (type d), SM7 (type e), and SM72 (type c) are type strains obtained from R. Facklam at the Centers for Disease Control (Atlanta, Georgia). Strains 142, 327, and 351 are *H. influenzae* type e isolates, and 15 strains 134, 219, 256, and 501 are *H. influenzae* type f isolates obtained from H. Kayhty (Finnish National Public Health Institute, Helsinki). Strain Rd (type d) and the 15 nontypable isolates examined by Southern analysis have been described previously (Alexander et al., *J. Exp. Med.* **83**:345-359 (1951); Barencamp et al., *Infect. Immun.* **60**:1302-1313 (1992)). *E. coli* DH5 $\alpha$  is a nonadherent laboratory 20 strain that was originally obtained from Gibco BRL. *E. coli* strain BL21(DE3) was a gift from F.W. Studier and contains a single copy of the T7 RNA polymerase gene under the control of the *lac* regulatory system (Studier, F.W., and B.A. Moffatt. 1986. Use of bacteriophage T7 RNA polymerase to direct high-level expression of cloned genes. *J. Mol. Biol.* **189**:113-130.). Plasmid pT7-7 was provided by S. Tabor and contains the T7 RNA polymerase promoter *f10*, a ribosome-bindingsite, and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site (Tabor, S., and C.L. Richardson. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Natl. Acad. Sci. USA.* **82**:1074-1078.). pUC19 is a high-copy-number plasmid 25 Proc. Natl. Acad. Sci. USA. **82**:1074-1078.).

that has been previously described (Yanish-Perron et al., Gene 33:103-119 (1985)). pDC400 is a pUC19 derivative that harbors the *H. influenzae* strain C54 surface fibril locus and is sufficient to promote *in vitro* adherence by laboratory strains of *E. coli* (St. Geme, J.W. III, and D. Cutter, 1995. Evidence that surface fibrils expressed by *Haemophilus influenzae* type b promote attachment to human epithelial cells. Mol. Microbiol. 15:77-85.). pHMW8-5 is a pT7-7 derivative that contains the *H. influenzae* strain 11 *hia* locus and also promotes adherence by nonadherent laboratory strains of *E. coli* (Barenkamp, S.J., and J.W. St. Geme, III. Identification of a second family of high molecular weight adhesion proteins expressed by nontypable *Haemophilus influenzae*. Mol. Microbiol., in press.). pHMW8-6 contains the *H. influenzae* *hia* locus interrupted by a kanamycin cassette (Barenkamp, S.J., and J.W. St. Geme, III. Identification of a second family of high molecular weight adhesion proteins expressed by nontypable *Haemophilus influenzae*. Mol. Microbiol., in press.). pUC4K served as the source of the kanamycin-resistance gene that was used as a probe in Southern analysis (Vieira, J., and J. Messing, 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene. 19:259-268.).

#### Culture conditions

*H. influenzae* strains were grown on chocolate agar supplemented with 1% Isovitale X, on brain heart infusion agar supplemented with hemin and NAD (BHI-DB agar), or in brain heart infusion broth supplemented with hemin and NAD (BHIs) (Anderson, P., R.B. Johnston, Jr., and D.H. Smith, 1972. Human serum activity against *Haemophilus influenzae* type b. J. Clin. Invest. 51:31-38.). These strains were stored at -80°C in brain heart infusion broth with 25% glycerol. *E. coli* strains were grown on Luria Bertani (LB) agar or in LB broth and were stored at -80°C in LB broth with 50% glycerol. For *H. influenzae*, kanamycin was used in a

concentration of 25 mg/ml. Antibiotic concentrations for *E. coli* included the following: ampicillin or carbenicillin 100 mg/ml and kanamycin 50 mg/ml.

#### Induction of plasmid-encoded proteins

To identify plasmid-encoded proteins, the bacteriophage T7 expression vector pT7-7 was employed and the relevant pT7-7 derivatives were transformed into *E. coli* BL21(DE3). Activation of the T7 promoter was achieved by inducing expression of T7 RNA polymerase with isopropyl-β-D-thiogalactopyranoside (final concentration, 1 mM). After induction for 30 minutes at 37°C, rifampicin was added to a final concentration of 200 mg/ml. Thirty minutes later, 1 ml of culture was pulsed with 50 mCi of trans-[<sup>35</sup>S]-label (ICN, Irvine, Calif.) for 5 minutes. Bacteria were harvested, and whole cell lysates were resuspended in Laemmli buffer for analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 7.5% acrylamide gels (Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London). 227:680-685.).

Autoradiography was performed with Kodak XAR-5 film.

#### Recombinant DNA methods

DNA ligations, restriction endonuclease digestions, and gel electrophoresis were performed according to standard techniques (Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Plasmids were introduced into *E. coli* strains by either chemical transformation or electroporation, as described (Dower, W.J., J.F. Miller, and C.W. Ragsdale. 1988. High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res.* 16:6127-6145..Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Transformation in *H. influenzae* was performed using the MIV method of Herriott et al. (Herriott,

R.M., E.M. Meyer, and M. Vogt. 1970. Defined nongrowth media for stage II competence in *Haemophilus influenzae*. *J. Bacteriol.* **101**:517-524.).

#### **Adherence assays**

Adherence assays were performed with tissue culture cells which were seeded into wells of 24-well tissue culture plates as previously described (St. Geme et al., *Infect. Immun.* **58**:4036-4044 (1991)). Adherence was measured after incubating bacteria with epithelial monolayers for 30 minutes as described (St. Geme, J.W.III, S. Falkow, and S.J. Barenkamp. 1993. High-molecular-weight proteins of nontypable *Haemophilus influenzae* mediate attachment to human epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **90**:2875-2879.). Tissue culture cells included Chang epithelial cells (Wong-Kilbourn derivative, clone 1-5c-4 (human conjunctiva)) (ATCC CCL 20.2), KB cells (human oral epidermoid carcinoma) (ATCC CCL 17), HEp-2 cells (human laryngeal epidermoid carcinoma) (ATCC CCL 23), A549 cells (human lung carcinoma) (ATCC CCL 185), Intestine 407 cells (human embryonic intestine) (ATCC CCL 6), HeLa cells (human cervical epitheloid carcinoma) (ATCC CCL 2), ME-180 cells (human cervical epidermoid carcinoma) (ATCC HTB 33), HEC-IB cells (human endometrium) (ATCC HTB 113), and CHO-K1 cells (Chinese hamster ovary) (ATCC CCL 61). Chang, KB, Intestine 407, HeLa, and HEC-IB cells were maintained in modified Eagle medium with Earle's salts and non-essential amino acids. HEp-2 cells were maintained in Dulbecco's modified Eagle medium. A549 cells and CHO-K1 cells in F12 medium (Ham), and ME-180 cells in McCoy5A medium. All media were supplemented with 10% heat-inactivated fetal bovine serum.

#### **Southern analysis**

Southern blotting was performed using high stringency conditions as previously described (St. Geme, J.W.III, and S. Falkow. 1991. Loss of capsule expression by

*Haemophilus influenzae* type b results in enhanced adherence to and invasion of human cells. Infect. Immun. 59:1325-1333.).

#### Microscopy

Samples of epithelial cells with associated bacteria were stained with Giemsa stain and examined by light microscopy as described (St. Geme, J.W.III, and S. Falkow, S. 1990. *Haemophilus influenzae* adheres to and enters cultured human epithelial cells. Infect. Immun. 58:4036-4044.).

For negative-staining electron microscopy, bacteria were stained with 0.5% aqueous uranyl acetate (St. Geme, J.W.III, and S. Falkow. 1991. Loss of capsule expression by *Haemophilus influenzae* type b results in enhanced adherence to and invasion of human cells. Infect. Immun. 59:1325-1333.) and examined using a Zeiss 10A microscope.

The previous study indicated that laboratory *E. coli* strains harboring the plasmid pDC400 were capable of efficient attachment to cultured human epithelial cells (St. Geme, J.W.III, and D. Cutter. 1995. Evidence that surface fibrils expressed by *Haemophilus influenzae* type b promote attachment to human epithelial cells. Mol. Microbiol. 15:77-85.). Subcloning studies and transposon mutagenesis indicated that the relevant coding region of pDC400 was present within an 8.3 kb  $\lambda$ bal fragment (St. Geme, J.W.III, and D. Cutter. 1995. Evidence that surface fibrils expressed by *Haemophilus influenzae* type b promote attachment to human epithelial cells. Mol. Microbiol. 15:77-85.) (Figure 7). To confirm this conclusion, in the present study this  $\lambda$ bal fragment was subcloned into pT7-7, generating plasmids designated pDC601 and pDC602, which contained the insert in opposite orientations (Figure 7). As predicted, expression of these plasmids in *E. coli* DH5 $\alpha$  was associated with a capacity for high level *in vitro* attachment (Table 1).

**Table 1. Adherence to Chang conjunctival cells.**

<u>Strain</u>	<u>ADHERENCE (% inoculum)*</u>
DH5 $\alpha$ /pT7-7	0.4 $\pm$ 0.1
DH5 $\alpha$ /pDC400	25.3 $\pm$ 1.2
5 DH5 $\alpha$ /pDC601	54.3 $\pm$ 7.5
DH5 $\alpha$ /pDC602	55.5 $\pm$ 4.3
10 C54b <sup>p</sup>	98.7 $\pm$ 9.5
C54-HA1::kan <sup>b</sup>	1.5 $\pm$ 0.2
15 C54-Tn400.23 <sup>c</sup>	3.3 $\pm$ 0.4

10 \*Adherence was measured in a 30 minute assay and was calculated by dividing the number of adherent bacteria by the number of inoculated bacteria. Values are the mean  $\pm$  SEM of measurements made in triplicate from representative experiments.

15 <sup>b</sup>Strain C54-HA1::kan was constructed by transforming C54b<sup>p</sup> with linearized pHMW8-6, which contains the HA1 gene with an intragenic kanamycin cassette.

20 <sup>c</sup>Strain C54-Tn400.23 contains a mini-Tn10 kan element in the *hsf* locus (St. Geme et al., Mol. Microbiol. 15:77-85 (1995)).

25 To determine the direction of transcription and identify plasmid-encoded proteins, pDC601 and pDC602 were subsequently introduced into *E. coli* BL21(DE3), producing BL21(DE3)/pDC601 and BL21(DE3)/pDC602, respectively. As a negative control, pT7-7 was also transformed into BL21(DE3). The T7 promoter in these three strains was induced with IPTG, and induced proteins were detected using trans-[<sup>35</sup>S]-label. As shown in Figure 8, induction of BL21(DE3)/pDC601 resulted in expression of a large protein over 200 kDa in size along with several slightly smaller proteins, which presumably represent degradation products. In contrast, when BL21(DE3)/pDC602 and BL21(DE3)/pT7-7 were induced, there

was no expression of these proteins. This experiment indicated that the genetic material contained in the 8.3 kb *Xba*I fragment is transcribed from left to right as shown in Figure 7 and suggested that a single long open reading frame may be present.

5       **Nucleotide sequencing**

Nucleotide sequence was determined using a Sequenase kit and double-stranded plasmid template. DNA fragments were subcloned into pUC19 and sequenced along both strands by primer walking. DNA sequence analysis was performed using the Genetics Computer Group (GCG) software package from the University of Wisconsin (Devereux, J., P. Haeblerli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12**:387-395.) Sequence similarity searches were carried out using the BLAST program of the National Center for Biotechnology Information (Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basis local alignment search tool. *J. Mol. Biol.* **215**:403-410.).

Sequencing of the 8.3 kb *Xba*I fragment revealed a 7059 bp gene, which is designated for literature purposes as *hsf* for *Haemophilus* surface fibrils, and is referred to herein as HA2. This gene encodes a 2353-amino acid polypeptide, referred to as Hsf or HA2, with a calculated molecular mass of 243.8 kDa, which is similar in size to the observed protein species detected after induction of BL21(DE3)/pDC601. The *HA2* gene has a GC content of 42.8%, somewhat greater than the published estimate of 38-39% for the whole genome (Fleischmann et al., 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science*, **269**: 496-512.. Kilian, M. 1976. A taxonomic study of the genus *Haemophilus*, with proposal of a new species. *J. Gen. Microbiol.* **93**:9-62.). A putative ribosomal binding site with the sequence AAGGTA begins 13 base pairs upstream of the presumed initiation codon. A sequence similar to a *rho*-independent

transcription terminator is present beginning 20 nucleotides beyond the stop codon and contains interrupted inverted repeats with the potential for forming a hairpin structure containing a loop of two bases and a stem of 11 bases. Of note, a string of 29 thymines spans the region from 149 to 121 nucleotides upstream of *HA2*.

5 **Homology to *HA1*/*HA1***

The nontypable *H. influenzae* nonpilus protein *HA1* protein (called *Hia* in the literature) promotes attachment to cultured human epithelial cells as outlined above. Comparison of the predicted amino acid sequence of *HA2* and the sequence of *HA1* revealed 81% similarity and 72% identity overall. As depicted in Figure 5, the two 10 sequences are highly conserved at their N-terminal and C-terminal ends, and both contain a Walker box nucleotide-binding motif. Interestingly, *HA1* is encoded by a 3.2 kb gene and is only 115-kDa. In this context, it is noteworthy that three separate stretches of *HA2* (corresponding to amino acids 174 to 608, 847 to 1291, and 1476 to 1914, respectively) show significant homology to the region of *HA1* 15 defined by amino acids 221 to 658 (Figure 5). Table 2 summarizes the level of similarity and identity between these three stretches of *HA2* and one another. The suggestion is that the larger size of *HA2* may relate in part to the presence of a repeated domain which is present in single copy in *HA1*.

Table 2. Percent similarity and percent identity between *HA2* repeats.

20	Percent Similarity/Percent Identity		
	HA2 174-608 <sup>a</sup>	HA2 847-1291 <sup>a</sup>	HA2 1476-1914 <sup>a</sup>
HA2 174-608	*	65/53	76/60
HA2 847-1291		*	70/56
HA2 1476-1914			*

25 <sup>a</sup>Numbers correspond to amino acid residue positions in the full-length *HA2* (Hsf) protein.

To evaluate whether *HA1* and *HA2* are alleles of the same locus, a series of Southern blots were performed. Samples of chromosomal DNA from strains C54 and 11 were subjected to digestion with *Bgl*II, *Cla*I and either *Pst*I or *Xba*I. Resulting DNA fragments were separated by agarose electrophoresis and transferred bidirectionally to nitrocellulose membranes. One membrane was probed with a 3.3 kb internal fragment of the *HA2* gene (Figure 7), and the other membrane was probed with a 1.6 kb intragenic fragment of the *HA1* gene. As shown in Figure 9, both probes 5 recognized exactly the same chromosomal fragments.

To obtain additional evidence that the *HA2* and *HA1* genes are homologs, the 10 inactivation of *HA2* by transformation of *H. influenzae* strain C54b:p with insertionally inactivated *HA1* was attempted. The plasmid pHMW8-6 (Barenkamp, S.J., and J.W. St. Geme, III. Identification of a second family of high molecular weight 15 adhesion proteins expressed by nontypable *Haemophilus influenzae*. Mol. Microbiol., in press.), which contains the *HA1* gene with an intragenic kanamycin cassette, was linearized with *Nde*I and introduced into competent C54. Southern hybridization confirmed insertion of the kanamycin cassette into *HA2* (not shown). Furthermore, examination of the C54 mutant by negative staining transmission electron microscopy revealed the loss of surface fibrils (not shown). Consistent with these findings, the 20 mutant strain demonstrated minimal attachment to Chang conjunctival cells (Table 1).

In additional experiments, the cellular binding specificities conferred by the *HA2* 25 and *HA1* proteins were compared. As shown in Figure 10, DH5 $\alpha$ /pDC601 (expressing *HA2*) demonstrated high level attachment to Chang cells, KB cells, HeLa cells, and Intestine 407 cells, moderate level attachment to HEp-2 cells, and minimal attachment to HEC-1B cells, ME-180 cells, and CHO-K1 cells. DH5 $\alpha$  harboring pHMW8-5 (expressing *HA1*) showed virtually the same pattern of attachment.

Giemsa staining and subsequent examination by light microscopy confirmed these viable count adherence assay results.

#### **Homology to other bacterial extracellular proteins**

A protein sequence similarity search was performed with the HA2 predicted amino acid sequence using the BLAST network service of the National Center for Biotechnology Information (Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basis local alignment search tool. *J. Mol. Biol.* **215**:403-410.). This search revealed low-level sequence similarity to a series of other bacterial adherence factors, including HMW1 and HMW2 (the proteins previously identified as being important adhesins in HA1-deficient nontypable *H. influenzae* strains; (St. Geme, J.W.III. S. Falkow, and S.J. Barenkamp. 1993. High-molecular-weight proteins of nontypable *Haemophilus influenzae* mediate attachment to human epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **90**:2875-2879.), AIDA-I (an adhesion protein expressed by some diarrheagenic *E. coli* strains: Benz, I., and M.A. Schmidt. 1992. AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic *Escherichia coli* strain 2787 (O126:H27), is synthesized via a precursor molecule. *Mol. Microbiol.* **6**:1539-1546.), and Tsh (a hemagglutinin produced by an avian pathogenic *E. coli* strain: Provence, D. and R. Curtiss III. 1994. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. *Infect Immun.* **62**:1369-1380.). In addition, HA2 showed homology to SepA, a *Shigella flexneri* secreted protein that appears to play a role in tissue invasion (Benjelloun-Touimi, Z., P.J. Sansonetti, and C. Parsot. 1995. SepA, the major extracellular protein of *Shigella flexneri*: autonomous secretion and involvement in tissue invasion. *Mol. Microbiol.* **17**:123-135.). Alignment of HA2 with HMW1, HMW2, AIDA-I, Tsh, and SepA revealed a highly conserved N-terminal domain (Figure 11). In AIDA-I, Tsh, and SepA, this N-terminal extremity precedes a typical prokaryotic signal sequence (Benjelloun-Touimi, Z., P.J. Sansonetti, and C. Parsot. 1995. SepA, the major extracellular protein of *Shigella flexneri*: autonomous secretion

WO 96/30519

- 44 -

and involvement in tissue invasion. Mol. Microbiol. 17:123-135.). Similarly, in HA2 this conserved domain precedes a 26 amino acid segment that is characterized by a positively charged region, followed by a string of hydrophobic residues, and then alanine-glutamine-alanine.

5        **Presence of an HA2 homolog in other encapsulated and nonencapsulated strains**  
Previous work demonstrated that an HA2 homolog is present in *H. influenzae* type b strains M42 and Eagan (St. Geme, J.W.III, and D. Cutter. 1995. Evidence that surface fibrils expressed by *Haemophilus influenzae* type b promote attachment to human epithelial cells. Mol. Microbiol. 15:77-85.). To define the extent to which the HA2 locus is shared by other type b strains, a panel of evolutionarily diverse type b isolates by Southern analysis were examined. Among these strains were six belonging to phylogenetic division I and four belonging to phylogenetic division II (Musser, J.M., J.S. Kroll, E.R. Moxon, and R.K. Selander. 1988. Evolutionary genetics of the encapsulated strains of *Haemophilus influenzae*. Proc. Natl. Acad. Sci. U.S.A. 85:7758-7762.). Chromosomal DNA was digested with *Bgl*II and then probed with the intragenic 3.3 kb fragment of the HA2 gene. As shown in Figure 12, all 10 strains showed hybridization. The universal presence among *H. influenzae* type b raised the question of the prevalence of this locus in other non-type b encapsulated *H. influenzae*. Southern analysis of a series of type a, c, d, e, and f isolates again demonstrated a homolog in all cases (Figure 13).

20        Recently Fleischmann et al. (Fleischmann R.D., et al.. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science. 269: 496-512.) reported the genome sequence of *H. influenzae* strain Rd, which was one of the two serotype d strains examined by Southern analysis. In accord with the Southern blotting results, search of the Rd genome revealed an open reading frame with striking sequence similarity to HA2. The Rd gene is 894 nucleotides in length and is predicted to encode a protein of 298 amino acids. Overall, the Rd locus is 70% identical to

the C54 *HA2* gene, and the Rd derived amino acid sequence is 62% identical and 75% similar to C54 *HA2*. Interestingly, the Rd open reading frame appears to be truncated due to a "premature" stop codon.

Previous experiments revealed that 13 of 15 nontypable strains lacking an  
5 HMW1/HMW2-related protein had evidence of an *HA1* homolog (Barenkamp, S.J., and J.W. St. Geme, III. Identification of a second family of high molecular weight adhesion proteins expressed by nontypable *Haemophilus influenzae*. *Mol. Microbiol.*, in press.). Consistent with the demonstration that *HA2* and *HA1* are homologous. Southern analysis of these 15 strains, probing with the 3.3 kb fragment of *hsf*,  
10 demonstrated hybridization in 12 of the same 13 (not shown).

#### **Chromosomal location of the *HA2* locus**

In earlier work, the *HA1* locus in nontypable strain 11 was found to be flanked upstream by an open reading frame with significant homology to *E. coli* exoribonuclease II (Barenkamp, S.J., and J.W. St. Geme, III. Identification of a second  
15 family of high molecular weight adhesion proteins expressed by nontypable *Haemophilus influenzae*. *Mol. Microbiol.*, in press.). Similarly, the *HA2* locus in strain C54 likewise is flanked on the 5' side by an open reading frame with similarity to *E. coli* exonuclease II. This gene terminates 357 base pairs before the *HA2* start codon and encodes a protein with a predicted amino acid sequence that is 61% similar  
20 and 33% identical at its C-terminal end to exoribonuclease II. Of note, the Rd *HA2* homolog is also flanked upstream by the exoribonuclease II locus.

#### **EXAMPLE 3**

##### **Cloning of HA3**

Recombinant phage containing the nontypable *Haemophilus* strain 32 *HA3* gene were  
25 isolated and characterized using methods modified slightly from those described

previously (Barenkamp and St. Geme, Molecular Microbiology 1996, in press). In brief, chromosomal DNA from strain 32 was prepared by a modification of the method of Marmur (Marmur, 1961). *Sau3A* partial restriction digests of the DNA were prepared fractionated on 0.7% agarose gels. Fractions containing DNA fragments prepared fractionated on 0.7% agarose gels. Fractions containing DNA fragments 5 in the 9- to 20- kbp range were pooled, and a library was prepared by ligation into  $\lambda$ EMBL3 arms. Ligation mixtures were packaged in vitro with Gigapack<sup>®</sup> (Stratagene, La Jolla, CA) and plate amplified in a P2 lysogen of *E. coli* LE392.

Lambda plaque screening was performed using a mixture of three PCR products derived from strain 32 chromosomal DNA. These PCR products were amplified using 10 primer pairs previously shown to amplify DNA segments at the 5' end of the strain 11 *HA1* gene. The primers were as follows:

Primer designation	strand	sequence
44P	positive	CCG TGC TTG CCC AAC ACG CTT
64P	positive	GCT GCC ACC TTG CAC AAC AAC
93G-2	positive	CTT TCA ATG CCA GAA AGT AGG
18T-1	negative	CTT CAA CCG TTG CGG ACA ACA

Each of the positive strand primers was used with the single negative strand primer to generate the three fragments used for probing the library.

The PCR products generated from strain 11 and strain 32 chromosomal DNA were 20 identical in size, suggesting that the nucleotide sequences of these chromosomal regions were similar in the two strains. Plaque screening was performed using standard methodology (Berger and Kimmel, 1987) at high stringency: final wash conditions were 65°C for 1 hour in buffer containing 2XSSC and 1% SDS. Positive plaques were identified by autoradiography, plaque purified and phage DNA was purified by standard methods. The same primer pairs used to generate the screening 25

probes were then used to localize the HA3 gene by amplifying various restriction fragments derived from the phage DNA. Once localized, the strain 32 HA3 gene and flanking DNA were sequenced using standard methods.

5 In order to construct strain 32 isogenic *Haemophilus influenzae* mutants deficient in expression of the HA3 gene, bacteria were made competent using the MIV (Herriott et al. 1970) and were transformed with linearized pHMW8-6, selecting for kanamycin resistance. Allelic exchange was confirmed by Southern analysis. The mutants that no longer expressed HA3 exhibited a marked decrease in binding to Chang epithelial cells, using the methods outlined above (data not shown).

10 Expression in non-adherent strains of *E. coli* did not result in adherence, although it has not been confirmed that the protein was actually expressed.

WO 96/30519

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Washington University

(ii) TITLE OF INVENTION: HAEMOPHILUS ADHESION PROTEINS

(iii) NUMBER OF SEQUENCES: 19

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3294 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGAACAAAA	TTTTAACGT	TATTTGGAAT	GTTGTGACTC	AAACTGGGT	TGTCGTATCT	60
GAACTCACTC	GCACCCACAC	CAAATGCGCC	TCCGCCACCG	TGGCGGTTGC	CGTATTGGCA	120
ACCCCTGTTGT	CCGCAACGGT	TGAGGCGAAC	AACAATACTC	CTGTTACGAA	TAAGTTGAAG	180
GCTTATGGCG	ATGCGAATT	TAATTCACT	AATAATTGCA	TAGCAGATGC	AGAAAAACAA	240
GTTCAAGAGG	CTTATAAAGG	TTTATTAAAT	CTAAATGAAA	AAAATGCGAG	TGATAAACTG	300
TTGGTGGAGG	ACAATACTGC	GGCGACCGTA	GGCAATTGCG	GTAAATTGGG	CTGGGTATTG	360
TCTAGCAAAA	ACGGCACAAG	GAACGAGAAA	AGCCAACAAG	TCAACATGC	GGATGAAGTG	420
TTGTTGAAG	GCAAAGGCGG	TGTGCAGGTT	ACTTCCACCT	CTGAAAACGG	CAAACACACC	480
ATTACCTTG	CTTAGCGAA	AGACCTTGGT	GTGAAAACGT	CGACTGTGAG	TGATACCTTA	540
ACGATTGGCG	GTGGTGCTGC	TGCAGGTGCT	ACAACAACAC	CGAAAGTGAA	TGTAACCTAGT	600
ACAACGTATG	GCTTGAAGTT	CGCTAAAGAT	GCTGCGGGTG	CTAATGGCGA	TACTACGGTT	660
CACTTGAATG	GTATTGGTT	AACCTTGACA	GACACGCTTG	TGGGTTCTCC	TGCTACTCAT	720
ATTGACGGAG	GAGATCAAAG	TACGCATTAC	ACTCGTGCAG	CAAGTATCAA	GGATGTCTTG	780
AATGCGGGTT	GGAATATCAA	GGGTGTTAAA	GCTGGCTCAA	CAACTGGTCA	ATCAGAAAAT	840
GTCGATTTG	TTCATACTTA	CGATACTGTT	GAGTTCTTGA	GTGCGGATAC	AGAGACCACG	900
ACTGTTACTG	TAGATAGCAA	AGAAAACGGT	AAGAGAACCG	AAGTTAAAAT	CGGTGCGAAG	960
ACTTCTGTTA	TCAAAGAAAA	AGACGGTAAG	TTATTTACTG	GAAAAGCTAA	CAAAGAGACA	1020
AATAAAGTTG	ATGGTGCTAA	CGCGACTGAA	GATGCAGACG	AAGGCAAAGG	CTTAGTGACT	1080
GCGAAAGATG	TGATTGACGC	AGTGAATAAG	ACTGGTTGGA	GAATTAAAAC	AACCGATGCT	1140
AATGGTCAA	ATGGCGACTT	CGCAACTGTT	GCATCAGGCA	CAAATGTAAC	CTTGCTAGT	1200
GGTAATGGTA	CAACTGCGAC	TGTAACATAAT	GGCACCGATG	GTATTACCGT	TAAGTATGAT	1260
GCGAAAGTTG	GCGACGGCTT	AAAAGTAGAT	GGCGATAAAA	TCGCTGCAGA	TACGACCGCA	1320
CTTACTGTGA	ATGATGGTAA	GAACGCTAAT	AATCCGAAAG	GTAAAGTGGC	TGATGTTGCT	1380
TCAACTGACG	AGAAGAAATT	GGTTACAGCA	AAAGGTTAG	TAACAGCCTT	AAACAGTCTA	1440
AGCTGGACTA	CAACTGCTGC	TGAGGCGGAC	GGTGGTACGC	TTGATGGAAA	TGCAAGTGAG	1500
CAAGAAGTTA	AAGCGGGCGA	TAAAGTAACC	TTTAAAGCAG	GCAAGAACTT	AAAAGTGAAA	1560
CAAGAGGGTG	CGAACCTTAC	TTATTCACTG	CAAGATGCTT	TAACAGGCTT	AACGAGCATT	1620
ACTTTAGGTA	CAGGAAATAA	TGGTGCAGAA	ACTGAAATCA	ACAAAGACGG	CTTAACCATC	1680

ACACCAGCAA	ATGGTGCAGG	TGCAAATAAT	GCAAACACCA	TCAGCGTAAC	CAAAGACGGC	1740
ATTAGTGCAGG	GCGGTCAGTC	GGTTAAAAAC	GTTGTGAGCG	GACTGAAGAA	ATTTGGTGAT	1800
GCGAATTCG	ATCCGCTGAC	TAGCTCCGCC	GACAACCTAA	CGAAACAAAA	TGACGATGCC	1860
TATAAAGGCT	TGACCAATT	GGATGAAAAA	GGTACAGACA	AGCAAACCTCC	AGTTGTTGCC	1920
GACAATACCG	CCGCAACCGT	GGGCGATTTG	CGCGGCTTGG	GCTGGGTCA	TTCTGCGGAC	1980
AAAACCACAG	GCGGCTCAAC	GGAATATCAC	GATCAAGTTC	GGAATGCGAA	CGAAGTGAAA	2040
TTCAAAAGCG	GCAACGGTAT	CAATGTTCC	GGTAAAACGG	TCAACGGTAG	GCGTGAAATT	2100
ACTTTGAAT	TGGCTAAAGG	TGAAGTGGTT	AAATCGAATG	AATTTACCGT	CAAAGAAACC	2160
AATGGAAAGG	AAACGAGCCT	GGTTAAAGTT	GGCGATAAAAT	ATTACAGCAA	AGAGGATATT	2220
GACTTAACAA	CAGGTCAAGCC	TAAATTAAAA	GATGGCAATA	CAGTTGCTGC	GAAATATCAA	2280
GATAAAGGTG	GCAAAGTCGT	TTCTGTAACG	GATAATACTG	AAGCTACCAT	AACCAACAAA	2340
GGTTCTGGCT	ATGTAACAGG	TAACCAAGTG	GCAGATGCGA	TTGCGAAATC	AGGCTTTGAG	2400
CTTGGCTTGG	CTGATGAAGC	TGATGCGAAA	CGGGCGTTTG	ATGATAAGAC	AAAAGCCTTA	2460
TCTGCTGGTA	CAACGGAAAT	TGTAAATGCC	CACGATAAAAG	TCCGTTTG	TAATGGTTTA	2520
AATAACCAAAG	TGAGCGCGC	AACGGTGGAA	AGCACCAGATG	CAAACGGCGA	TAAAGTGACC	2580
ACAACCTTG	TGAAAACCGA	TGTGGAATTG	CCTTTAACGC	AAATCTACAA	TACCGATGCA	2640
AACGGTAAGA	AAATCACTAA	AGTTGTCAA	GATGGGCAA	CTAAATGGTA	TGAACTGAAT	2700
GCTGACGGTA	CGGCTGATAT	GACCAAAGAA	GTTACCCCTCG	GTAACGTGGA	TTCAAGACGGC	2760
AAGAAAAGTTG	TGAAAGACAA	CGATGGCAAG	TGGTATCAGC	CCAAAGCTGA	CGGTACTGCG	2820
GATAAAACCA	AAGGCAGAGT	GAGCAATGAT	AAAGTTTCTA	CCGATGAAAA	ACACGTTGTC	2880
AGCCTTGATC	CAAATGATCA	ATCAAAGGT	AAAGGTGTG	TGATTGACAA	TGTGGCTAAT	2940
GGCGATATT	CTGCCACTTC	CACCGATGCG	ATTAACGGAA	GTCAGTTGTA	TGCTGTGGCA	3000
AAAGGGGTAA	CAAACCTTGC	TGGACAAGTG	AATAATCTG	AGGGCAAAGT	GAATAAAGTG	3060
GGCAAACGTG	CAGATGCAGG	TACAGCAAGT	GCATTAGCGG	CTTCACAGTT	ACCACAAGCC	3120
ACTATGCCAG	GTAAATCAAT	GGTTGCTATT	GCAGGGAAAGTA	GTTATCAAGG	TCAAATGGT	3180
TTAGCTATCG	GGGTATCAAG	AATTTCCGAT	AATGGCAAAG	TGATTATTG	CTTGTCAAGGC	3240
ACAACCAATA	GTCAAGGTAA	AACAGGGCTT	GCAGCAGGTG	TTGGTTACCA	GTGG	3294

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1098 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asn	Lys	Ile	Phe	Asn	Val	Ile	Trp	Asn	Val	Val	Thr	Gln	Thr	Trp
1							5			10				15	
Val	Val	Val	Ser	Glu	Leu	Thr	Arg	Thr	His	Thr	Lys	Cys	Ala	Ser	Ala
						20			25				30		
Thr	Val	Ala	Val	Ala	Val	Leu	Ala	Thr	Leu	Leu	Ser	Ala	Thr	Val	Glu
						35			40				45		
Ala	Asn	Asn	Asn	Thr	Pro	Val	Thr	Asn	Lys	Leu	Lys	Ala	Tyr	Gly	Asp
						50			55				60		
Ala	Asn	Phe	Asn	Phe	Thr	Asn	Asn	Ser	Ile	Ala	Asp	Ala	Glu	Lys	Gln
						65			70				75		80
Val	Gln	Glu	Ala	Tyr	Lys	Gly	Leu	Leu	Asn	Leu	Asn	Glu	Lys	Asn	Ala
						85			90				95		
Ser	Asp	Lys	Leu	Leu	Val	Glu	Asp	Asn	Thr	Ala	Ala	Thr	Val	Gly	Asn
						100			105				110		
Leu	Arg	Lys	Leu	Gly	Trp	Val	Leu	Ser	Ser	Lys	Asn	Gly	Thr	Arg	Asn
						115			120				125		
Glu	Lys	Ser	Gln	Gln	Val	Lys	His	Ala	Asp	Glu	Val	Leu	Phe	Glu	Gly
						130			135				140		
Lys	Gly	Gly	Val	Gln	Val	Thr	Ser	Thr	Ser	Glu	Asn	Gly	Lys	His	Thr
						145			150				155		160
Ile	Thr	Phe	Ala	Leu	Ala	Lys	Asp	Leu	Gly	Val	Lys	Thr	Ala	Thr	Val
						165			170				175		
Ser	Asp	Thr	Leu	Thr	Ile	Gly	Gly	Ala	Ala	Ala	Gly	Ala	Thr	Thr	
						180			185				190		
Thr	Pro	Lys	Val	Asn	Val	Thr	Ser	Thr	Thr	Asp	Gly	Leu	Lys	Phe	Ala
						195			200				205		
Lys	Asp	Ala	Ala	Gly	Ala	Asn	Gly	Asp	Thr	Thr	Val	His	Leu	Asn	Gly
						210			215				220		
Ile	Gly	Ser	Thr	Leu	Thr	Asp	Thr	Leu	Val	Gly	Ser	Pro	Ala	Thr	His
						225			230				235		240

52

Ile Asp Gly Gly Asp Gln Ser Thr His Tyr Thr Arg Ala Ala Ser Ile  
 245 250 255  
 Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Ala Gly  
 260 265 270  
 Ser Thr Thr Gly Gln Ser Glu Asn Val Asp Phe Val His Thr Tyr Asp  
 275 280 285  
 Thr Val Glu Phe Leu Ser Ala Asp Thr Glu Thr Thr Thr Val Thr Val  
 290 295 300  
 Asp Ser Lys Glu Asn Gly Lys Arg Thr Glu Val Lys Ile Gly Ala Lys  
 305 310 315 320  
 Thr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Ala  
 325 330 335  
 Asn Lys Glu Thr Asn Lys Val Asp Gly Ala Asn Ala Thr Glu Asp Ala  
 340 345 350  
 Asp Glu Gly Lys Gly Leu Val Thr Ala Lys Asp Val Ile Asp Ala Val  
 355 360 365  
 Asn Lys Thr Gly Trp Arg Ile Lys Thr Thr Asp Ala Asn Gly Gln Asn  
 370 375 380  
 Gly Asp Phe Ala Thr Val Ala Ser Gly Thr Asn Val Thr Phe Ala Ser  
 385 390 395 400  
 Gly Asn Gly Thr Thr Ala Thr Val Thr Asn Gly Thr Asp Gly Ile Thr  
 405 410 415  
 Val Lys Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Leu Asp Gly Asp  
 420 425 430  
 Lys Ile Ala Ala Asp Thr Thr Ala Leu Thr Val Asn Asp Gly Lys Asn  
 435 440 445  
 Ala Asn Asn Pro Lys Gly Lys Val Ala Asp Val Ala Ser Thr Asp Glu  
 450 455 460  
 Lys Lys Leu Val Thr Ala Lys Gly Leu Val Thr Ala Leu Asn Ser Leu  
 465 470 475 480  
 Ser Trp Thr Thr Ala Ala Glu Ala Asp Gly Gly Thr Leu Asp Gly  
 485 490 495  
 Asn Ala Ser Glu Gln Glu Val Lys Ala Gly Asp Lys Val Thr Phe Lys  
 500 505 510  
 Ala Gly Lys Asn Leu Lys Val Lys Gln Glu Gly Ala Asn Phe Thr Tyr  
 515 520 525  
 Ser Leu Gln Asp Ala Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Thr  
 530 535 540

53

Gly Asn Asn Gly Ala Lys Thr Glu Ile Asn Lys Asp Gly Leu Thr Ile  
 545 550 555 560

Thr Pro Ala Asn Gly Ala Gly Ala Asn Asn Ala Asn Thr Ile Ser Val  
 565 570 575

Thr Lys Asp Gly Ile Ser Ala Gly Gly Gln Ser Val Lys Asn Val Val  
 580 585 590

Ser Gly Leu Lys Lys Phe Gly Asp Ala Asn Phe Asp Pro Leu Thr Ser  
 595 600 605

Ser Ala Asp Asn Leu Thr Lys Gln Asn Asp Asp Ala Tyr Lys Gly Leu  
 610 615 620

Thr Asn Leu Asp Glu Lys Gly Thr Asp Lys Gln Thr Pro Val Val Ala  
 625 630 635 640

Asp Asn Thr Ala Ala Thr Val Gly Asp Leu Arg Gly Leu Gly Trp Val  
 645 650 655

Ile Ser Ala Asp Lys Thr Thr Gly Gly Ser Thr Glu Tyr His Asp Gln  
 660 665 670

Val Arg Asn Ala Asn Glu Val Lys Phe Lys Ser Gly Asn Gly Ile Asn  
 675 680 685

Val Ser Gly Lys Thr Val Asn Gly Arg Arg Glu Ile Thr Phe Glu Leu  
 690 695 700

Ala Lys Gly Glu Val Val Lys Ser Asn Glu Phe Thr Val Lys Glu Thr  
 705 710 715 720

Asn Gly Lys Glu Thr Ser Leu Val Lys Val Gly Asp Lys Tyr Tyr Ser  
 725 730 735

Lys Glu Asp Ile Asp Leu Thr Thr Gly Gln Pro Lys Leu Lys Asp Gly  
 740 745 750

Asn Thr Val Ala Ala Lys Tyr Gln Asp Lys Gly Lys Val Val Ser  
 755 760 765

Val Thr Asp Asn Thr Glu Ala Thr Ile Thr Asn Lys Gly Ser Gly Tyr  
 770 775 780

Val Thr Gly Asn Gln Val Ala Asp Ala Ile Ala Lys Ser Gly Phe Glu  
 785 790 795 800

Leu Gly Leu Ala Asp Glu Ala Asp Ala Lys Arg Ala Phe Asp Asp Lys  
 805 810 815

Thr Lys Ala Leu Ser Ala Gly Thr Thr Glu Ile Val Asn Ala His Asp  
 820 825 830

Lys Val Arg Phe Ala Asn Gly Leu Asn Thr Lys Val Ser Ala Ala Thr  
 835 840 845

Val Glu Ser Thr Asp Ala Asn Gly Asp Lys Val Thr Thr Thr Phe Val  
 850 855 860  
 Lys Thr Asp Val Glu Leu Pro Leu Thr Gln Ile Tyr Asn Thr Asp Ala  
 865 870 875 880  
 Asn Gly Lys Lys Ile Thr Lys Val Val Lys Asp Gly Gln Thr Lys Trp  
 885 890 895  
 Tyr Glu Leu Asn Ala Asp Gly Thr Ala Asp Met Thr Lys Glu Val Thr  
 900 905 910  
 Leu Gly Asn Val Asp Ser Asp Gly Lys Lys Val Val Lys Asp Asn Asp  
 915 920 925  
 Gly Lys Trp Tyr His Ala Lys Ala Asp Gly Thr Ala Asp Lys Thr Lys  
 930 935 940  
 Gly Glu Val Ser Asn Asp Lys Val Ser Thr Asp Glu Lys His Val Val  
 945 950 955 960  
 Ser Leu Asp Pro Asn Asp Gln Ser Lys Gly Lys Gly Val Val Ile Asp  
 965 970 975  
 Asn Val Ala Asn Gly Asp Ile Ser Ala Thr Ser Thr Asp Ala Ile Asn  
 980 985 990  
 Gly Ser Gln Leu Tyr Ala Val Ala Lys Gly Val Thr Asn Leu Ala Gly  
 995 1000 1005  
 Gln Val Asn Asn Leu Glu Gly Lys Val Asn Lys Val Gly Lys Arg Ala  
 1010 1015 1020  
 Asp Ala Gly Thr Ala Ser Ala Leu Ala Ala Ser Gln Leu Pro Gln Ala  
 1025 1030 1035 1040  
 Thr Met Pro Gly Lys Ser Met Val Ala Ile Ala Gly Ser Ser Tyr Gln  
 1045 1050 1055  
 Gly Gln Asn Gly Leu Ala Ile Gly Val Ser Arg Ile Ser Asp Asn Gly  
 1060 1065 1070  
 Lys Val Ile Ile Arg Leu Ser Gly Thr Thr Asn Ser Gln Gly Lys Thr  
 1075 1080 1085  
 Gly Val Ala Ala Gly Val Gly Tyr Gln Trp  
 1090 1095

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 7291 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 163..7221

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTNTTTTTC TTATTTTTT TTTTTTTTT TTGAGGCTAA ACTTTTNGNA	60
AAATATCACT TTTTATTCT CCAAATATAG AATAGAATAC GCACGATTTC ACTAAGAAAA	120
GTATATTTAT CATTAATTAA ATTAAATATA AGGTAAATAA AA ATG AAC AAA ATT	174
Met Asn Lys Ile	
1	
TTT AAC GTT ATT TGG AAT GTT ATG ACT CAA ACT TGG GTT GTC GTA TCT	222
Phe Asn Val Ile Trp Asn Val Met Thr Gln Thr Trp Val Val Val Ser	
5 10 15 20	
GAA CTC ACT CGC ACC CAC ACC AAA CGC GCC TCC GCA ACC GTG GAG ACC	270
Glu Leu Thr Arg Thr His Thr Lys Arg Ala Ser Ala Thr Val Glu Thr	
25 30 35	
GCC GTA TTG GCG ACA CTG TTG TTT GCA ACG GTT CAG GCG AAT GCT ACC	318
Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln Ala Asn Ala Thr	
40 45 50	
GAT GAA GAT GAA GAG TTA GAC CCC GTA GTA CGC ACT GCT CCC GTG TTG	366
Asp Glu Asp Glu Glu Leu Asp Pro Val Val Arg Thr Ala Pro Val Leu	
55 60 65	
AGC TTC CAT TCC GAT AAA GAA GGC ACG GGA GAA AAA GAA GTT ACA GAA	414
Ser Phe His Ser Asp Lys Glu Gly Thr Gly Glu Lys Glu Val Thr Glu	
70 75 80	
AAT TCA AAT TGG GGA ATA TAT TTC GAC AAT AAA GGA GTA CTA AAA GCC	462
Asn Ser Asn Trp Gly Ile Tyr Phe Asp Asn Lys Gly Val Leu Lys Ala	
85 90 95 100	
GGA GCA ATC ACC CTC AAA GCC GGC GAC AAC CTG AAA ATC AAA CAA AAC	510
Gly Ala Ile Thr Leu Lys Ala Gly Asp Asn Leu Lys Ile Lys Gln Asn	
105 110 115	
ACC GAT GAA AGC ACC AAT GCC AGT AGC TTC ACC TAC TCG CTG AAA AAA	558
Thr Asp Glu Ser Thr Asn Ala Ser Ser Phe Thr Tyr Ser Leu Lys Lys	
120 125 130	
GAC CTC ACA GAT CTG ACC AGT GTT GCA ACT GAA AAA TTA TCG TTT GGC	606
Asp Leu Thr Asp Leu Thr Ser Val Ala Thr Glu Lys Leu Ser Phe Gly	
135 140 145	
GCA AAC GGC GAT AAA GTT GAT ATT ACC AGT GAT GCA AAT GGC TTG AAA	654
Ala Asn Gly Asp Lys Val Asp Ile Thr Ser Asp Ala Asn Gly Leu Lys	
150 155 160	

TTG GCG AAA ACA GGT AAC GGA AAT GTT CAT TTG AAT GGT TTG GAT TCA	702
Leu Ala Lys Thr Gly Asn Gly Asn Val His Leu Asn Gly Leu Asp Ser	
165 170 175 180	
ACT TTG CCT GAT GCG GTA ACG AAT ACA GGT GTG TTA AGT TCA TCA AGT	750
Thr Leu Pro Asp Ala Val Thr Asn Thr Gly Val Leu Ser Ser Ser	
185 190 195	
TTT ACA CCT AAT GAT GTT GAA AAA ACA AGA GCT GCA ACT GTT AAA GAT	798
Phe Thr Pro Asn Asp Val Glu Lys Thr Arg Ala Ala Thr Val Lys Asp	
200 205 210	
GTG TTA AAT GCA GGT TGG AAC ATT AAA GGT GCT AAA ACT GCT GGA GGT	846
Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Ala Lys Thr Ala Gly Gly	
215 220 225	
AAT GTT GAG AGT GTT GAT TTA GTG TCC GCT TAT AAT GTT GAA TTT	894
Asn Val Glu Ser Val Asp Leu Val Ser Ala Tyr Asn Asn Val Glu Phe	
230 235 240	
ATT ACA GGC GAT AAA AAC ACG CTT GAT GTT GTA TTA ACA GCT AAA GAA	942
Ile Thr Gly Asp Lys Asn Thr Leu Asp Val Val Leu Thr Ala Lys Glu	
245 250 255 260	
AAC GGT AAA ACA ACC GAA GTG AAA TTC ACA CCG AAA ACC TCT GTT ATC	990
Asn Gly Lys Thr Thr Glu Val Lys Phe Thr Pro Lys Thr Ser Val Ile	
265 270 275	
AAA GAA AAA GAC GGT AAG TTA TTT ACT GGA AAA GAG AAT AAC GAC ACA	1038
Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Glu Asn Asn Asp Thr	
280 285 290	
AAT AAA GTT ACA AGT AAC ACG GCG ACT GAT AAT ACA GAT GAG GGT AAT	1086
Asn Lys Val Thr Ser Asn Thr Ala Thr Asp Asn Thr Asp Glu Gly Asn	
295 300 305	
GGC TTA GTC ACT GCA AAA GCT GTG ATT GAT GCT GTG AAC AAG GCT GGT	1134
Gly Leu Val Thr Ala Lys Ala Val Ile Asp Ala Val Asn Lys Ala Gly	
310 315 320	
TGG AGA GTT AAA ACA ACT ACT GCT AAT GGT CAA AAT GGC GAC TTC GCA	1182
Trp Arg Val Lys Thr Thr Ala Asn Gly Gln Asn Gly Asp Phe Ala	
325 330 335 340	
ACT GTT GCG TCA GGC ACA AAT GTA ACC TTT GAA AGT GGC GAT GGT ACA	1230
Thr Val Ala Ser Gly Thr Asn Val Thr Phe Glu Ser Gly Asp Gly Thr	
345 350 355	
ACA GCG TCA GTA ACT AAA GAT ACT AAC GGC AAT GGC ATC ACT GTT AAG	1278
Thr Ala Ser Val Thr Lys Asp Thr Asn Gly Asn Gly Ile Thr Val Lys	
360 365 370	
TAC GAC GCG AAA GTT GGC GAC GGC TTG AAA TTT GAT AGC GAT AAA AAA	1326
Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Phe Asp Ser Asp Lys Lys	
375 380 385	

ATC GTT GCA GAT ACG ACC GCA CTT ACT GTG ACA GGT GGT AAG GTA GCT Ile Val Ala Asp Thr Thr Ala Leu Thr Val Thr Gly Gly Lys Val Ala 390 395 400	1374
GAA ATT GCT AAA GAA GAT GAC AAG AAA AAA CTT GTT AAT GCA GGC GAT Glu Ile Ala Lys Glu Asp Asp Lys Lys Lys Leu Val Asn Ala Gly Asp 405 410 415 420	1422
TTG GTA ACA GCT TTA GGT AAT CTA AGT TGG AAA GCA AAA GCT GAG GCT Leu Val Thr Ala Leu Gly Asn Leu Ser Trp Lys Ala Lys Ala Glu Ala 425 430 435	1470
GAT ACT GAT GGT GCG CTT GAG GGG ATT TCA AAA GAC CAA GAA GTC AAA Asp Thr Asp Gly Ala Leu Glu Gly Ile Ser Lys Asp Gln Glu Val Lys 440 445 450	1518
GCA GGC GAA ACG GTA ACC TTT AAA GCG GGC AAG AAC TTA AAA GTG AAA Ala Gly Glu Thr Val Thr Phe Lys Ala Gly Lys Asn Leu Lys Val Lys 455 460 465	1566
CAG GAT GGT GCG AAC TTT ACT TAT TCA CTG CAA GAT GCT TTA ACG GGT Gln Asp Gly Ala Asn Phe Thr Tyr Ser Leu Gln Asp Ala Leu Thr Gly 470 475 480	1614
TTA ACG AGC ATT ACT TTA GGT GGT ACA ACT AAT GGC GGA AAT GAT GCG Leu Thr Ser Ile Thr Leu Gly Gly Thr Thr Asn Gly Gly Asn Asp Ala 485 490 495 500	1662
AAA ACC GTC ATC AAC AAA GAC GGT TTA ACC ATC ACG CCA GCA GGT AAT Lys Thr Val Ile Asn Lys Asp Gly Leu Thr Ile Thr Pro Ala Gly Asn 505 510 515	1710
GGC GGT ACG ACA GGT ACA AAC ACC ATC AGC GTA ACC AAA GAT GGC ATT Gly Gly Thr Thr Gly Thr Asn Thr Ile Ser Val Thr Lys Asp Gly Ile 520 525 530	1758
AAA GCA GGT AAT AAA GCT ATT ACT AAT GTT GCG AGT GGT TTA AGA GCT Lys Ala Gly Asn Lys Ala Ile Thr Asn Val Ala Ser Gly Leu Arg Ala 535 540 545	1806
TAT GAC GAT GCG AAT TTT GAT GTT TTA AAT AAC TCT GCA ACT GAT TTA Tyr Asp Asp Ala Asn Phe Asp Val Leu Asn Asn Ser Ala Thr Asp Leu 550 555 560	1854
AAT AGA CAC GTT GAA GAT GCT TAT AAA GGT TTA TTA AAT CTA AAT GAA Asn Arg His Val Glu Asp Ala Tyr Lys Gly Leu Leu Asn Leu Asn Glu 565 570 575 580	1902
AAA AAT GCA AAT AAA CAA CCG TTG GTG ACT GAC AGC ACG GCG GCG ACT Lys Asn Ala Asn Lys Gln Pro Leu Val Thr Asp Ser Thr Ala Ala Thr 585 590 595	1950
GTA GGC GAT TTA CGT AAA TTG GGT TGG GTA GTA TCA ACC AAA AAC GGT Val Gly Asp Leu Arg Lys Leu Gly Trp Val Val Ser Thr Lys Asn Gly 600 605 610	1998

WO 96/30519

58

ACG AAA GAA GAA AGC AAT CAA GTT AAA CAA GCT GAT GAA GTC CTC TTT	2046
Thr Lys Glu Glu Ser Asn Gln Val Lys Gln Ala Asp Glu Val Leu Phe	
615 620 625	
ACC GGA GCC GGT GCT GCT ACG GTT ACT TCC AAA TCT GAA AAC GGT AAA	2094
Thr Gly Ala Gly Ala Ala Thr Val Thr Ser Lys Ser Glu Asn Gly Lys	
630 635 640	
CAT ACG ATT ACC GTT AGT GTG GCT GAA ACT AAA GCG GAT TGC GGT CTT	2142
His Thr Ile Thr Val Ser Val Ala Glu Thr Lys Ala Asp Cys Gly Leu	
645 650 655 660	
GAA AAA GAT GGC GAT ACT ATT AAG CTC AAA GTG GAT AAT CAA AAC ACT	2190
Glu Lys Asp Gly Asp Thr Ile Lys Leu Lys Val Asp Asn Gln Asn Thr	
665 670 675	
GAT AAT GTT TTA ACT GTT GGT AAT AAT GGT ACT GCT GTC ACT AAA GGT	2238
Asp Asn Val Leu Thr Val Gly Asn Asn Gly Thr Ala Val Thr Lys Gly	
680 685 690	
GGC TTT GAA ACT GTT AAA ACT GGA GCG ACT GAT GCA GAT CGC GGT AAA	2286
Gly Phe Glu Thr Val Lys Thr Gly Ala Thr Asp Ala Asp Arg Gly Lys	
695 700 705	
GTA ACT GTA AAA GAT GCT ACT GCT AAT GAC GCT GAT AAG AAA GTC GCA	2334
Val Thr Val Lys Asp Ala Thr Ala Asn Asp Ala Asp Lys Lys Val Ala	
710 715 720	
ACT GTA AAA GAT GTT GCA ACC GCA ATT AAT AGT GCG GCG ACT TTT GTG	2382
Thr Val Lys Asp Val Ala Thr Ala Ile Asn Ser Ala Ala Thr Phe Val	
725 730 735 740	
AAA ACA GAG AAT TTA ACT ACC TCT ATT GAT GAA GAT AAT CCT ACA GAT	2430
Lys Thr Glu Asn Leu Thr Thr Ser Ile Asp Glu Asp Asn Pro Thr Asp	
745 750 755	
AAC GGC AAA GAT GAC GCA CTT AAA GCG GGC GAT ACC TTA ACC TTT AAA	2478
Asn Gly Lys Asp Asp Ala Leu Lys Ala Gly Asp Thr Leu Thr Phe Lys	
760 765 770	
GCA GGT AAA AAC CTG AAA GTT AAA CGT GAT GGA AAA AAT ATT ACT TTT	2526
Ala Gly Lys Asn Leu Lys Val Lys Arg Asp Gly Lys Asn Ile Thr Phe	
775 780 785	
GAC TTG GCG AAA AAC CTT GAG GTG AAA ACT GCG AAA GTG AGT GAT ACT	2574
Asp Leu Ala Lys Asn Leu Glu Val Lys Thr Ala Lys Val Ser Asp Thr	
790 795 800	
TTA ACG ATT GGC GGG AAT ACA CCT ACA GGT GGC ACT ACT GCG ACG CCA	2622
Leu Thr Ile Gly Gly Asn Thr Pro Thr Gly Gly Thr Thr Ala Thr Pro	
805 810 815 820	
AAA GTG AAT ATT ACT AGC ACG GCT GAT GGT TTG AAT TTT GCA AAA GAA	2670
Lys Val Asn Ile Thr Ser Thr Ala Asp Gly Leu Asn Phe Ala Lys Glu	
825 830 835	

ACA GCC GAT GCC TCG GGT TCT AAG AAT GTT TAT TTG AAA GGT ATT GCG	2718																																																																																																										
Thr Ala Asp Ala Ser Gly Ser Lys Asn Val Tyr Leu Lys Gly Ile Ala																																																																																																											
840	845	850		ACA ACT TTA ACT GAG CCA AGC GCG GGA GCG AAG TCT TCA CAC GTT GAT	2766	Thr Thr Leu Thr Glu Pro Ser Ala Gly Ala Lys Ser Ser His Val Asp		855	860	865		TTA AAT GTG GAT GCG ACG AAA AAA TCC AAT GCA GCA AGT ATT GAA GAT	2814	Leu Asn Val Asp Ala Thr Lys Lys Ser Asn Ala Ala Ser Ile Glu Asp		870	875	880		GTA TTG CGC GCA GGT TGG AAT ATT CAA GGT AAT GGT AAT ATT GTT GAT	2862	Val Leu Arg Ala Gly Trp Asn Ile Gln Gly Asn Gly Asn Asn Val Asp		885	890	895	900	TAT GTA GCG ACG TAT GAC ACA GTA AAC TTT ACC GAT GAC AGC ACA GGT	2910	Tyr Val Ala Thr Tyr Asp Thr Val Asn Phe Thr Asp Asp Ser Thr Gly		905	910	915		ACA ACA ACG GTA ACC GCA ACC CAA AAA GCA GAT GGC AAA GGT GCT GAC	2958	Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly Lys Gly Ala Asp		920	925	930		GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006	Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys		935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060
850																																																																																																											
ACA ACT TTA ACT GAG CCA AGC GCG GGA GCG AAG TCT TCA CAC GTT GAT	2766																																																																																																										
Thr Thr Leu Thr Glu Pro Ser Ala Gly Ala Lys Ser Ser His Val Asp																																																																																																											
855	860	865		TTA AAT GTG GAT GCG ACG AAA AAA TCC AAT GCA GCA AGT ATT GAA GAT	2814	Leu Asn Val Asp Ala Thr Lys Lys Ser Asn Ala Ala Ser Ile Glu Asp		870	875	880		GTA TTG CGC GCA GGT TGG AAT ATT CAA GGT AAT GGT AAT ATT GTT GAT	2862	Val Leu Arg Ala Gly Trp Asn Ile Gln Gly Asn Gly Asn Asn Val Asp		885	890	895	900	TAT GTA GCG ACG TAT GAC ACA GTA AAC TTT ACC GAT GAC AGC ACA GGT	2910	Tyr Val Ala Thr Tyr Asp Thr Val Asn Phe Thr Asp Asp Ser Thr Gly		905	910	915		ACA ACA ACG GTA ACC GCA ACC CAA AAA GCA GAT GGC AAA GGT GCT GAC	2958	Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly Lys Gly Ala Asp		920	925	930		GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006	Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys		935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060								
865																																																																																																											
TTA AAT GTG GAT GCG ACG AAA AAA TCC AAT GCA GCA AGT ATT GAA GAT	2814																																																																																																										
Leu Asn Val Asp Ala Thr Lys Lys Ser Asn Ala Ala Ser Ile Glu Asp																																																																																																											
870	875	880		GTA TTG CGC GCA GGT TGG AAT ATT CAA GGT AAT GGT AAT ATT GTT GAT	2862	Val Leu Arg Ala Gly Trp Asn Ile Gln Gly Asn Gly Asn Asn Val Asp		885	890	895	900	TAT GTA GCG ACG TAT GAC ACA GTA AAC TTT ACC GAT GAC AGC ACA GGT	2910	Tyr Val Ala Thr Tyr Asp Thr Val Asn Phe Thr Asp Asp Ser Thr Gly		905	910	915		ACA ACA ACG GTA ACC GCA ACC CAA AAA GCA GAT GGC AAA GGT GCT GAC	2958	Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly Lys Gly Ala Asp		920	925	930		GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006	Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys		935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																
880																																																																																																											
GTA TTG CGC GCA GGT TGG AAT ATT CAA GGT AAT GGT AAT ATT GTT GAT	2862																																																																																																										
Val Leu Arg Ala Gly Trp Asn Ile Gln Gly Asn Gly Asn Asn Val Asp																																																																																																											
885	890	895	900	TAT GTA GCG ACG TAT GAC ACA GTA AAC TTT ACC GAT GAC AGC ACA GGT	2910	Tyr Val Ala Thr Tyr Asp Thr Val Asn Phe Thr Asp Asp Ser Thr Gly		905	910	915		ACA ACA ACG GTA ACC GCA ACC CAA AAA GCA GAT GGC AAA GGT GCT GAC	2958	Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly Lys Gly Ala Asp		920	925	930		GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006	Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys		935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																								
895	900																																																																																																										
TAT GTA GCG ACG TAT GAC ACA GTA AAC TTT ACC GAT GAC AGC ACA GGT	2910																																																																																																										
Tyr Val Ala Thr Tyr Asp Thr Val Asn Phe Thr Asp Asp Ser Thr Gly																																																																																																											
905	910	915		ACA ACA ACG GTA ACC GCA ACC CAA AAA GCA GAT GGC AAA GGT GCT GAC	2958	Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly Lys Gly Ala Asp		920	925	930		GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006	Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys		935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																
915																																																																																																											
ACA ACA ACG GTA ACC GCA ACC CAA AAA GCA GAT GGC AAA GGT GCT GAC	2958																																																																																																										
Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly Lys Gly Ala Asp																																																																																																											
920	925	930		GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006	Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys		935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																								
930																																																																																																											
GTT AAA ATC GGT GCG AAA ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA	3006																																																																																																										
Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys																																																																																																											
935	940	945		CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054	Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val		950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																
945																																																																																																											
CTG TTT ACA GGC AAA GAC CTG AAA GAT GCG AAT ATT GGT GCA ACC GTT	3054																																																																																																										
Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn Gly Ala Thr Val																																																																																																											
950	955	960		AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102	Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys		965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																								
960																																																																																																											
AGT GAA GAT GAT GGC AAA GAC ACC GGC ACA GGC TTA GTT ACT GCA AAA	3102																																																																																																										
Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu Val Thr Ala Lys																																																																																																											
965	970	975	980	ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150	Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu		985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																																
975	980																																																																																																										
ACT GTG ATT GAT GCA GTA AAT AAA AGC GGT TGG AGG GTA ACC GGT GAG	3150																																																																																																										
Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg Val Thr Gly Glu																																																																																																											
985	990	995		GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198	Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala		1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																																								
995																																																																																																											
GGC GCG ACT GCC GAA ACC GGT GCA ACC GCC GTG AAT GCG GGT AAC GCT	3198																																																																																																										
Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn Ala Gly Asn Ala																																																																																																											
1000	1005	1010		GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246	Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala		1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																																																
1010																																																																																																											
GAA ACC GTT ACA TCA GGC ACG AGC GTG AAC TTC AAA AAC GGC AAT GCG	3246																																																																																																										
Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys Asn Gly Asn Ala																																																																																																											
1015	1020	1025		ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294	Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr		1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																																																								
1025																																																																																																											
ACC ACA GCG ACC GTA AGC AAA GAT AAT GGC AAC ATC AAT GTC AAA TAC	3294																																																																																																										
Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile Asn Val Lys Tyr																																																																																																											
1030	1035	1040		GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342	Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile		1045	1050	1055	1060																																																																																																
1040																																																																																																											
GAT GTA AAT GTT GGT GAC GGC TTG AAG ATT GGC GAT GAC AAA AAA ATC	3342																																																																																																										
Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp Asp Lys Lys Ile																																																																																																											
1045	1050	1055	1060																																																																																																								
1055	1060																																																																																																										

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GTT GCA GAC ACG ACC ACA CTT ACT GTA ACA GGT GGT AAG GTG TCT GTT	3390
Val Ala Asp Thr Thr Thr Leu Thr Val Thr Gly Gly Lys Val Ser Val	
1065 1070 1075	
CCT GCT GGT GCT AAT AGT GTT AAT AAC AAT AAG AAA CTT GTT AAT GCA	3438
Pro Ala Gly Ala Asn Ser Val Asn Asn Asn Lys Lys Leu Val Asn Ala	
1080 1085 1090	
GAG GGT TTA GCG ACT GCT TTA AAC AAC CTA AGC TGG ACG GCA AAA GCC	3486
Glu Gly Leu Ala Thr Ala Leu Asn Asn Leu Ser Trp Thr Ala Lys Ala	
1095 1100 1105	
GAT AAA TAT GCA GAT GGC GAG TCA GAG GGC GAA ACC GAC CAA GAA GTC	3534
Asp Lys Tyr Ala Asp Gly Glu Ser Glu Gly Glu Thr Asp Gln Glu Val	
1110 1115 1120	
AAA GCA GGC GAC AAA GTA ACC TTT AAA GCA GGC AAG AAC TTA AAA GTG	3582
Lys Ala Gly Asp Lys Val Thr Phe Lys Ala Gly Lys Asn Leu Lys Val	
1125 1130 1135 1140	
AAA CAG TCT GAA AAA GAC TTT ACT TAT TCA CTG CAA GAC ACT TTA ACA	3630
Lys Gln Ser Glu Lys Asp Phe Thr Tyr Ser Leu Gln Asp Thr Leu Thr	
1145 1150 1155	
GGC TTA ACG AGC ATT ACT TTA GGT GGT ACA GCT AAT GGC AGA AAT GAT	3678
Gly Leu Thr Ser Ile Thr Leu Gly Thr Ala Asn Gly Arg Asn Asp	
1160 1165 1170	
ACG GGA ACC GTC ATC AAC AAA GAC GGC TTA ACC ATC ACG CTG GCA AAT	3726
Thr Gly Thr Val Ile Asn Lys Asp Gly Leu Thr Ile Thr Leu Ala Asn	
1175 1180 1185	
GGT GCT GCG GCA GGC ACA GAT GCG TCT AAC GGA AAC ACC ATC AGT GTA	3774
Gly Ala Ala Ala Gly Thr Asp Ala Ser Asn Gly Asn Thr Ile Ser Val	
1190 1195 1200	
ACC AAA GAC GGC ATT AGT GCG GGT AAT AAA GAA ATT ACC AAT GTT AAG	3822
Thr Lys Asp Gly Ile Ser Ala Gly Asn Lys Glu Ile Thr Asn Val Lys	
1205 1210 1215 1220	
AGT GCT TTA AAA ACC TAT AAA GAT ACT CAA AAC ACT GCA GAT GAA ACA	3870
Ser Ala Leu Lys Thr Tyr Lys Asp Thr Gln Asn Thr Ala Asp Glu Thr	
1225 1230 1235	
CAA GAT AAA GAG TTC CAC GCC GTT AAA AAC GCA AAT GAA GTT GAG	3918
Gln Asp Lys Glu Phe His Ala Ala Val Lys Asn Ala Asn Glu Val Glu	
1240 1245 1250	
TTC GTG GGT AAA AAC GGT GCA ACC GTG TCT GCA AAA ACT GAT AAC AAC	3966
Phe Val Gly Lys Asn Gly Ala Thr Val Ser Ala Lys Thr Asp Asn Asn	
1255 1260 1265	
GGA AAA CAT ACT GTA ACG ATT GAT GTT GCA GAA GCC AAA GTT GGT GAT	4014
Gly Lys His Thr Val Thr Ile Asp Val Ala Glu Ala Lys Val Gly Asp	
1270 1275 1280	

GGT CTT GAA AAA GAT ACT GAC GGC AAG ATT AAA CTC AAA GTA GAT AAT 4062  
 Gly Leu Glu Lys Asp Thr Asp Gly Lys Ile Lys Leu Lys Val Asp Asn  
 1285 1290 1295 1300  
 ACA GAT GGG AAT AAT CTA TTA ACC GTT GAT GCA ACA AAA GGT GCA TCC 4110  
 Thr Asp Gly Asn Asn Leu Leu Thr Val Asp Ala Thr Lys Gly Ala Ser  
 1305 1310 1315  
 GTT GCC AAG GGC GAG TTT AAT GCC GTA ACA ACA GAT GCA ACT ACA GCC 4158  
 Val Ala Lys Gly Glu Phe Asn Ala Val Thr Thr Asp Ala Thr Thr Ala  
 1320 1325 1330  
 CAA GGC ACA AAT GCC AAT GAG CGC GGT AAA GTG GTT GTC AAG GGT TCA 4206  
 Gln Gly Thr Asn Ala Asn Glu Arg Gly Lys Val Val Val Lys Gly Ser  
 1335 1340 1345  
 AAT GGT GCA ACT GCT ACC GAA ACT GAC AAG AAA AAA GTG GCA ACT GTT 4254  
 Asn Gly Ala Thr Ala Thr Glu Thr Asp Lys Lys Val Ala Thr Val  
 1350 1355 1360  
 GGC GAC GTT GCT AAA GCG ATT AAC GAC GCA GCA ACT TTC GTG AAA GTG 4302  
 Gly Asp Val Ala Lys Ala Ile Asn Asp Ala Ala Thr Phe Val Lys Val  
 1365 1370 1375 1380  
 GAA AAT GAC GAC AGT GCT ACG ATT GAT GAT AGC CCA ACA GAT GAT GGC 4350  
 Glu Asn Asp Asp Ser Ala Thr Ile Asp Asp Ser Pro Thr Asp Asp Gly  
 1385 1390 1395  
 GCA AAT GAT GCT CTC AAA GCA GGC GAC ACC TTG ACC TTA AAA GCG GGT 4398  
 Ala Asn Asp Ala Leu Lys Ala Gly Asp Thr Leu Thr Leu Lys Ala Gly  
 1400 1405 1410  
 AAA AAC TTA AAA GTT AAA CGT GAT GGT AAA AAT ATT ACT TTT GCC CTT 4446  
 Lys Asn Leu Lys Val Lys Arg Asp Gly Lys Asn Ile Thr Phe Ala Leu  
 1415 1420 1425  
 GCG AAC GAC CTT AGT GTA AAA AGC GCA ACC GTT AGC GAT AAA TTA TCG 4494  
 Ala Asn Asp Leu Ser Val Lys Ser Ala Thr Val Ser Asp Lys Leu Ser  
 1430 1435 1440  
 CTT GGT ACA AAC GGC AAT AAA GTC AAT ATC ACA AGC GAC ACC AAA GGC 4542  
 Leu Gly Thr Asn Gly Asn Lys Val Asn Ile Thr Ser Asp Thr Lys Gly  
 1445 1450 1455 1460  
 TTG AAC TTC GCT AAA GAT AGT AAG ACA GGC GAT GAT GCT AAT ATT CAC 4590  
 Leu Asn Phe Ala Lys Asp Ser Lys Thr Gly Asp Asp Ala Asn Ile His  
 1465 1470 1475  
 TTA AAT GGC ATT GCT TCA ACT TTA ACT GAT ACA TTG TTA AAT AGT GGT 4638  
 Leu Asn Gly Ile Ala Ser Thr Leu Thr Asp Thr Leu Leu Asn Ser Gly  
 1480 1485 1490  
 GCG ACA ACC AAT TTA GGT GGT AAT GGT ATT ACT GAT AAC GAG AAA AAA 4686  
 Ala Thr Thr Asn Leu Gly Gly Asn Gly Ile Thr Asp Asn Glu Lys Lys  
 1495 1500 1505

CGC GCG GCG AGC GTT AAA GAT GTC TTG AAT GCG GGT TGG AAT GTT CGT 4734  
 Arg Ala Ala Ser Val Lys Asp Val Leu Asn Ala Gly Trp Asn Val Arg  
 1510 1515 1520

GGT GTT AAA CCG GCA TCT GCA AAT AAT CAA GTG GAG AAT ATC GAC TTT 4782  
 Gly Val Lys Pro Ala Ser Ala Asn Asn Gln Val Glu Asn Ile Asp Phe  
 1525 1530 1535 1540

GTA GCA ACC TAC GAC ACA GTG GAC TTT GTT AGT GGA GAT AAA GAC ACC 4830  
 Val Ala Thr Tyr Asp Thr Val Asp Phe Val Ser Gly Asp Lys Asp Thr  
 1545 1550 1555

ACG AGT GTA ACT GTT GAA AGT AAA GAT AAT GGC AAG AGA ACC GAA GTT 4878  
 Thr Ser Val Thr Val Glu Ser Lys Asp Asn Gly Lys Arg Thr Glu Val  
 1560 1565 1570

AAA ATC GGT GCG AAG ACT TCT GTT ATC AAA GAC CAC AAC GGC AAA CTG 4926  
 Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His Asn Gly Lys Leu  
 1575 1580 1585

TTT ACA GGC AAA GAG CTG AAG GAT GCT AAC AAT AAT GGC GTA ACT GTT 4974  
 Phe Thr Gly Lys Glu Leu Lys Asp Ala Asn Asn Asn Gly Val Thr Val  
 1590 1595 1600

ACC GAA ACC GAC GGC AAA GAC GAG GGT AAT GGT TTA GTG ACT GCA AAA 5022  
 Thr Glu Thr Asp Gly Lys Asp Glu Gly Asn Gly Leu Val Thr Ala Lys  
 1605 1610 1615 1620

GCT GTG ATT GAT GCC GTG AAT AAG GCT GGT TGG AGA GTT AAA ACA ACA 5070  
 Ala Val Ile Asp Ala Val Asn Lys Ala Gly Trp Arg Val Lys Thr Thr  
 1625 1630 1635

GGT GCT AAT GGT CAG AAT GAT GAC TTC GCA ACT GTT GCG TCA GGC ACA 5118  
 Gly Ala Asn Gly Gln Asn Asp Asp Phe Ala Thr Val Ala Ser Gly Thr  
 1640 1645 1650

AAT GTA ACC TTT GCT GAT GGT AAT GGC ACA ACT GCC GAA GTA ACT AAA 5166  
 Asn Val Thr Phe Ala Asp Gly Asn Gly Thr Thr Ala Glu Val Thr Lys  
 1655 1660 1665

GCA AAC GAC GGT AGT ATT ACT GTT AAA TAC AAT GTT AAA GTG GCT GAT 5214  
 Ala Asn Asp Gly Ser Ile Thr Val Lys Tyr Asn Val Lys Val Ala Asp  
 1670 1675 1680

GGC TTA AAA CTA GAC GGC GAT AAA ATC GTT GCA GAC ACG ACC GTA CTT 5262  
 Gly Leu Lys Leu Asp Gly Asp Lys Ile Val Ala Asp Thr Thr Val Leu  
 1685 1690 1695 1700

ACT GTG GCA GAT GGT AAA GTT ACA GCT CCG AAT AAT GGC GAT GGT AAG 5310  
 Thr Val Ala Asp Gly Lys Val Thr Ala Pro Asn Asn Gly Asp Gly Lys  
 1705 1710 1715

AAA TTT GTT GAT GCA AGT GGT TTA GCG GAT GCG TTA AAT AAA TTA AGC 5358  
 Lys Phe Val Asp Ala Ser Gly Leu Ala Asp Ala Leu Asn Lys Leu Ser  
 1720 1725 1730

TGG ACG GCA ACT GCT GGT AAA GAA GGC ACT GGT GAA GTT GAT CCT GCA Trp Thr Ala Thr Ala Gly Lys Glu Gly Thr Gly Glu Val Asp Pro Ala 1735 1740 1745	5406
AAT TCA GCA GGG CAA GAA GTC AAA GCG GGC GAC AAA GTA ACC TTT AAA Asn Ser Ala Gly Gln Glu Val Lys Ala Gly Asp Lys Val Thr Phe Lys 1750 1755 1760	5454
GCC GGC GAC AAC CTG AAA ATC AAA CAA AGC GGC AAA GAC TTT ACC TAC Ala Gly Asp Asn Leu Lys Ile Lys Gln Ser Gly Lys Asp Phe Thr Tyr 1765 1770 1775 1780	5502
TCG CTG AAA AAA GAG CTG AAA GAC CTG ACC AGC GTA GAG TTC AAA GAC Ser Leu Lys Lys Glu Leu Lys Asp Leu Thr Ser Val Glu Phe Lys Asp 1785 1790 1795	5550
GCA AAC GGC GGT ACA GGC AGT GAA AGC ACC AAG ATT ACC AAA GAC GGC Ala Asn Gly Gly Thr Gly Ser Glu Ser Thr Lys Ile Thr Lys Asp Gly 1800 1805 1810	5598
TTG ACC ATT ACG CCG GCA AAC GGT GCG GGT GCG GCA GGT GCA AAC ACT Leu Thr Ile Thr Pro Ala Asn Gly Ala Ala Gly Ala Asn Thr 1815 1820 1825	5646
GCA AAC ACC ATT AGC GTA ACC AAA GAT GGC ATT AGC GCG GGT AAT AAA Ala Asn Thr Ile Ser Val Thr Lys Asp Gly Ile Ser Ala Gly Asn Lys 1830 1835 1840	5694
GCA GTT ACA AAC GTT GTG AGC GGA CTG AAG AAA TTT GGT GAT GGT CAT Ala Val Thr Asn Val Val Ser Gly Leu Lys Lys Phe Gly Asp Gly His 1845 1850 1855 1860	5742
ACG TTG GCA AAT GGC ACT GTT GCT GAT TTT GAA AAG CAT TAT GAC AAT Thr Leu Ala Asn Gly Thr Val Ala Asp Phe Glu Lys His Tyr Asp Asn 1865 1870 1875	5790
GCC TAT AAA GAC TTG ACC AAT TTG GAT GAA AAA GGC GCG GAT AAT AAT Ala Tyr Lys Asp Leu Thr Asn Leu Asp Glu Lys Gly Ala Asp Asn Asn 1880 1885 1890	5838
CCG ACT GTT GCC GAC AAT ACC GCT GCA ACC GTG GGC GAT TTG CGC GGC Pro Thr Val Ala Asp Asn Thr Ala Ala Thr Val Gly Asp Leu Arg Gly 1895 1900 1905	5886
TTG GGC TGG GTC ATT TCT GCG GAC AAA ACC ACA GGC GAA CCC AAT CAG Leu Gly Trp Val Ile Ser Ala Asp Lys Thr Thr Gly Glu Pro Asn Gln 1910 1915 1920	5934
GAA TAC AAC GCG CAA GTG CGT AAC GCC AAT GAA GTG AAA TTC AAG AGC Glu Tyr Asn Ala Gln Val Arg Asn Ala Asn Glu Val Lys Phe Lys Ser 1925 1930 1935 1940	5982
GGC AAC GGT ATC AAT GTT TCC GGT AAA ACA TTG AAC GGT ACG CGC GTG Gly Asn Gly Ile Asn Val Ser Gly Lys Thr Leu Asn Gly Thr Arg Val 1945 1950 1955	6030

WO 96/30519

64

ATT ACC TTT GAA TTG GCT AAA GGC GAA GTG GTT AAA TCG AAT GAA TTT Ile Thr Phe Glu Leu Ala Lys Gly Glu Val Val Lys Ser Asn Glu Phe 1960 1965 1970	6078
ACC GTT AAG AAT GCC GAT GGT TCG GAA ACG AAC TTG GTT AAA GTT GGC Thr Val Lys Asn Ala Asp Gly Ser Glu Thr Asn Leu Val Lys Val Gly 1975 1980 1985	6126
GAT ATG TAT TAC AGC AAA GAG GAT ATT GAC CCG GCA ACC AGT AAA CCG Asp Met Tyr Tyr Ser Lys Glu Asp Ile Asp Pro Ala Thr Ser Lys Pro 1990 1995 2000	6174
ATG ACA GGT AAA ACT GAA AAA TAT AAG GTT GAA AAC GGC AAA GTC GTT Met Thr Gly Lys Thr Glu Lys Tyr Lys Val Glu Asn Gly Lys Val Val 2005 2010 2015 2020	6222
TCT GCT AAC GGC AGC AAG ACC GAA GTT ACC CTA ACC AAC AAA GGT TCC Ser Ala Asn Gly Ser Lys Thr Glu Val Thr Leu Thr Asn Lys Gly Ser 2025 2030 2035	6270
GGC TAT GTA ACA GGT AAC CAA GTG GCT GAT GCG ATT GCG AAA TCA GGC Gly Tyr Val Thr Gly Asn Gln Val Ala Asp Ala Ile Ala Lys Ser Gly 2040 2045 2050	6318
TTT GAG CTT GGT TTG GCT GAT GCG GCA GAA GCT GAA AAA GCC TTT GCA Phe Glu Leu Gly Leu Ala Asp Ala Ala Glu Ala Lys Ala Phe Ala 2055 2060 2065	6366
GAA AGC GCA AAA GAC AAG CAA TTG TCT AAA GAT AAA GCG GAA ACT GTA Glu Ser Ala Lys Asp Lys Gln Leu Ser Lys Asp Ala Glu Thr Val 2070 2075 2080	6414
AAT GCC CAC GAT AAA GTC CGT TTT GCT AAT GGT TTA AAT ACC AAA GTG Asn Ala His Asp Lys Val Arg Phe Ala Asn Gly Leu Asn Thr Lys Val 2085 2090 2095 2100	6462
AGC GCG GCA ACG GTG GAA AGC ACT GAT GCA AAC GGC GAT AAA GTG ACC Ser Ala Ala Thr Val Glu Ser Thr Asp Ala Asn Gly Asp Lys Val Thr 2105 2110 2115	6510
ACA ACC TTT GTG AAA ACC GAT GTG GAA TTG CCT TTA ACG CAA ATC TAC Thr Thr Phe Val Lys Thr Asp Val Glu Leu Pro Leu Thr Gln Ile Tyr 2120 2125 2130	6558
AAT ACC GAT GCA AAC GGT AAT AAG ATC GTT AAA AAA GCT GAC GGA AAA Asn Thr Asp Ala Asn Gly Asn Lys Ile Val Lys Lys Ala Asp Gly Lys 2135 2140 2145	6606
TGG TAT GAA CTG AAT GCT GAT GGT ACG GCG AGT AAC AAA GAA GTG ACA Trp Tyr Glu Leu Asn Ala Asp Gly Thr Ala Ser Asn Lys Glu Val Thr 2150 2155 2160	6654
CTT GGT AAC GTG GAT GCA AAC GGT AAG AAA GTT GTG AAA GTA ACC GAA Leu Gly Asn Val Asp Ala Asn Gly Lys Lys Val Val Lys Val Thr Glu 2165 2170 2175 2180	6702

AAT GGT GCG GAT AAG TGG TAT TAC ACC AAT GCT GAC GGT GCT GCG GAT Asn Gly Ala Asp Lys Trp Tyr Tyr Thr Asn Ala Asp Gly Ala Ala Asp 2185 2190 2195	6750
AAA ACC AAA GGC GAA GTG AGC AAT GAT AAA GTT TCT ACC GAT GAA AAA Lys Thr Lys Gly Glu Val Ser Asn Asp Lys Val Ser Thr Asp Glu Lys 2200 2205 2210	6798
CAC GTT GTC CGC CTT GAT CCG AAC AAT CAA TCG AAC GGC AAA GGC GTG His Val Val Arg Leu Asp Pro Asn Asn Gln Ser Asn Gly Lys Gly Val 2215 2220 2225	6846
GTC ATT GAC AAT GTG GCT AAT GGC GAA ATT TCT GCC ACT TCC ACC GAT Val Ile Asp Asn Val Ala Asn Gly Glu Ile Ser Ala Thr Ser Thr Asp 2230 2235 2240	6894
GGC ATT AAC GGA AGT CAG TTG TAT GCC GTG GCA AAA GGG GTA ACA AAC Ala Ile Asn Gly Ser Gln Leu Tyr Ala Val Ala Lys Gly Val Thr Asn 2245 2250 2255 2260	6942
CTT GCT GGA CAA GTG AAT AAT CTT GAG GGC AAA GTG AAT AAA GTG GGC Leu Ala Gly Gln Val Asn Asn Leu Glu Gly Lys Val Asn Lys Val Gly 2265 2270 2275	6990
AAA CGT GCA GAT GCA GGT ACA GCA AGT GCA TTA GCG GCT TCA CAG TTA Lys Arg Ala Asp Ala Gly Thr Ala Ser Ala Leu Ala Ala Ser Gln Leu 2280 2285 2290	7038
CCA CAA GCC ACT ATG CCA GGT AAA TCA ATG GTT GCT ATT GCG GGA AGT Pro Gln Ala Thr Met Pro Gly Lys Ser Met Val Ala Ile Ala Gly Ser 2295 2300 2305	7086
AGT TAT CAA GGT CAA AAT GGT TTA GCT ATC GGG GTA TCA AGA ATT TCC Ser Tyr Gln Gly Gln Asn Gly Leu Ala Ile Gly Val Ser Arg Ile Ser 2310 2315 2320	7134
GAT AAT GGC AAA GTG ATT ATT CGC TTG TCA GGC ACA ACC AAT AGT CAA Asp Asn Gly Lys Val Ile Ile Arg Leu Ser Gly Thr Thr Asn Ser Gln 2325 2330 2335 2340	7182
GGT AAA ACA GGC GTT GCA GCA GGT GTT GGT TAC CAG TGG TAAAGTTGG Gly Lys Thr Gly Val Ala Ala Gly Val Gly Tyr Gln Trp 2345 2350	7231
ATTATCTCTC TTAAAAAGCG GCATTTGCCG CTTTTTTAT GGGTGGCTAT TATGTATCGT	7291

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2353 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

WO 96/30519

66

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Met Thr Gln Thr Trp  
 1 5 10 15

Val Val Val Ser Glu Leu Thr Arg Thr His Thr Lys Arg Ala Ser Ala  
 20 25 30

Thr Val Glu Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln  
 35 40 45

Ala Asn Ala Thr Asp Glu Asp Glu Glu Leu Asp Pro Val Val Arg Thr  
 50 55 60

Ala Pro Val Leu Ser Phe His Ser Asp Lys Glu Gly Thr Gly Glu Lys  
 65 70 75 80

Glu Val Thr Glu Asn Ser Asn Trp Gly Ile Tyr Phe Asp Asn Lys Gly  
 85 90 95

Val Leu Lys Ala Gly Ala Ile Thr Leu Lys Ala Gly Asp Asn Leu Lys  
 100 105 110

Ile Lys Gln Asn Thr Asp Glu Ser Thr Asn Ala Ser Ser Phe Thr Tyr  
 115 120 125

Ser Leu Lys Lys Asp Leu Thr Asp Leu Thr Ser Val Ala Thr Glu Lys  
 130 135 140

Leu Ser Phe Gly Ala Asn Gly Asp Lys Val Asp Ile Thr Ser Asp Ala  
 145 150 155 160

Asn Gly Leu Lys Leu Ala Lys Thr Gly Asn Gly Asn Val His Leu Asn  
 165 170 175

Gly Leu Asp Ser Thr Leu Pro Asp Ala Val Thr Asn Thr Gly Val Leu  
 180 185 190

Ser Ser Ser Phe Thr Pro Asn Asp Val Glu Lys Thr Arg Ala Ala  
 195 200 205

Thr Val Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Ala Lys  
 210 215 220

Thr Ala Gly Gly Asn Val Glu Ser Val Asp Leu Val Ser Ala Tyr Asn  
 225 230 235 240

Asn Val Glu Phe Ile Thr Gly Asp Lys Asn Thr Leu Asp Val Val Leu  
 245 250 255

Thr Ala Lys Glu Asn Gly Lys Thr Thr Glu Val Lys Phe Thr Pro Lys  
 260 265 270

Thr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Glu  
 275 280 285

Asn Asn Asp Thr Asn Lys Val Thr Ser Asn Thr Ala Thr Asp Asn Thr  
290 295 300

Asp Glu Gly Asn Gly Leu Val Thr Ala Lys Ala Val Ile Asp Ala Val  
305 310 315 320

Asn Lys Ala Gly Trp Arg Val Lys Thr Thr Ala Asn Gly Gln Asn  
325 330 335

Gly Asp Phe Ala Thr Val Ala Ser Gly Thr Asn Val Thr Phe Glu Ser  
340 345 350

Gly Asp Gly Thr Thr Ala Ser Val Thr Lys Asp Thr Asn Gly Asn Gly  
355 360 365

Ile Thr Val Lys Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Phe Asp  
370 375 380

Ser Asp Lys Lys Ile Val Ala Asp Thr Thr Ala Leu Thr Val Thr Gly  
385 390 395 400

Gly Lys Val Ala Glu Ile Ala Lys Glu Asp Asp Lys Lys Lys Leu Val  
405 410 415

Asn Ala Gly Asp Leu Val Thr Ala Leu Gly Asn Leu Ser Trp Lys Ala  
420 425 430

Lys Ala Glu Ala Asp Thr Asp Gly Ala Leu Glu Gly Ile Ser Lys Asp  
435 440 445

Gln Glu Val Lys Ala Gly Glu Thr Val Thr Phe Lys Ala Gly Lys Asn  
450 455 460

Leu Lys Val Lys Gln Asp Gly Ala Asn Phe Thr Tyr Ser Leu Gln Asp  
465 470 475 480

Ala Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Gly Thr Thr Asn Gly  
485 490 495

Gly Asn Asp Ala Lys Thr Val Ile Asn Lys Asp Gly Leu Thr Ile Thr  
500 505 510

Pro Ala Gly Asn Gly Gly Thr Thr Gly Thr Asn Thr Ile Ser Val Thr  
515 520 525

Lys Asp Gly Ile Lys Ala Gly Asn Lys Ala Ile Thr Asn Val Ala Ser  
530 535 540

Gly Leu Arg Ala Tyr Asp Asp Ala Asn Phe Asp Val Leu Asn Asn Ser  
545 550 555 560

Ala Thr Asp Leu Asn Arg His Val Glu Asp Ala Tyr Lys Gly Leu Leu  
565 570 575

Asn Leu Asn Glu Lys Asn Ala Asn Lys Gln Pro Leu Val Thr Asp Ser  
580 585 590

WO 96/30519

68

Thr Ala Ala Thr Val Gly Asp Leu Arg Lys Leu Gly Trp Val Val Ser  
 595 600 605  
 Thr Lys Asn Gly Thr Lys Glu Glu Ser Asn Gln Val Lys Gln Ala Asp  
 610 615 620  
 Glu Val Leu Phe Thr Gly Ala Gly Ala Ala Thr Val Thr Ser Lys Ser  
 625 630 635 640  
 Glu Asn Gly Lys His Thr Ile Thr Val Ser Val Ala Glu Thr Lys Ala  
 645 650 655  
 Asp Cys Gly Leu Glu Lys Asp Gly Asp Thr Ile Lys Leu Lys Val Asp  
 660 665 670  
 Asn Gln Asn Thr Asp Asn Val Leu Thr Val Gly Asn Asn Gly Thr Ala  
 675 680 685  
 Val Thr Lys Gly Gly Phe Glu Thr Val Lys Thr Gly Ala Thr Asp Ala  
 690 695 700  
 Asp Arg Gly Lys Val Thr Val Lys Asp Ala Thr Ala Asn Asp Ala Asp  
 705 710 715 720  
 Lys Lys Val Ala Thr Val Lys Asp Val Ala Thr Ala Ile Asn Ser Ala  
 725 730 735  
 Ala Thr Phe Val Lys Thr Glu Asn Leu Thr Thr Ser Ile Asp Glu Asp  
 740 745 750  
 Asn Pro Thr Asp Asn Gly Lys Asp Asp Ala Leu Lys Ala Gly Asp Thr  
 755 760 765  
 Leu Thr Phe Lys Ala Gly Lys Asn Leu Lys Val Lys Arg Asp Gly Lys  
 770 775 780  
 Asn Ile Thr Phe Asp Leu Ala Lys Asn Leu Glu Val Lys Thr Ala Lys  
 785 790 795 800  
 Val Ser Asp Thr Leu Thr Ile Gly Gly Asn Thr Pro Thr Gly Thr  
 805 810 815  
 Thr Ala Thr Pro Lys Val Asn Ile Thr Ser Thr Ala Asp Gly Leu Asn  
 820 825 830  
 Phe Ala Lys Glu Thr Ala Asp Ala Ser Gly Ser Lys Asn Val Tyr Leu  
 835 840 845  
 Lys Gly Ile Ala Thr Thr Leu Thr Glu Pro Ser Ala Gly Ala Lys Ser  
 850 855 860  
 Ser His Val Asp Leu Asn Val Asp Ala Thr Lys Lys Ser Asn Ala Ala  
 865 870 875 880  
 Ser Ile Glu Asp Val Leu Arg Ala Gly Trp Asn Ile Gln Gly Asn Gly  
 885 890 895

Asn Asn Val Asp Tyr Val Ala Thr Tyr Asp Thr Val Asn Phe Thr Asp  
 900 905 910  
 Asp Ser Thr Gly Thr Thr Val Thr Val Thr Gln Lys Ala Asp Gly  
 915 920 925  
 Lys Gly Ala Asp Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp  
 930 935 940  
 His Asn Gly Lys Leu Phe Thr Gly Lys Asp Leu Lys Asp Ala Asn Asn  
 945 950 955 960  
 Gly Ala Thr Val Ser Glu Asp Asp Gly Lys Asp Thr Gly Thr Gly Leu  
 965 970 975  
 Val Thr Ala Lys Thr Val Ile Asp Ala Val Asn Lys Ser Gly Trp Arg  
 980 985 990  
 Val Thr Gly Glu Gly Ala Thr Ala Glu Thr Gly Ala Thr Ala Val Asn  
 995 1000 1005  
 Ala Gly Asn Ala Glu Thr Val Thr Ser Gly Thr Ser Val Asn Phe Lys  
 1010 1015 1020  
 Asn Gly Asn Ala Thr Thr Ala Thr Val Ser Lys Asp Asn Gly Asn Ile  
 1025 1030 1035 1040  
 Asn Val Lys Tyr Asp Val Asn Val Gly Asp Gly Leu Lys Ile Gly Asp  
 1045 1050 1055  
 Asp Lys Lys Ile Val Ala Asp Thr Thr Leu Thr Val Thr Gly Gly  
 1060 1065 1070  
 Lys Val Ser Val Pro Ala Gly Ala Asn Ser Val Asn Asn Asn Lys Lys  
 1075 1080 1085  
 Leu Val Asn Ala Glu Gly Leu Ala Thr Ala Leu Asn Asn Leu Ser Trp  
 1090 1095 1100  
 Thr Ala Lys Ala Asp Lys Tyr Ala Asp Gly Glu Ser Glu Gly Glu Thr  
 1105 1110 1115 1120  
 Asp Gln Glu Val Lys Ala Gly Asp Lys Val Thr Phe Lys Ala Gly Lys  
 1125 1130 1135  
 Asn Leu Lys Val Lys Gln Ser Glu Lys Asp Phe Thr Tyr Ser Leu Gln  
 1140 1145 1150  
 Asp Thr Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Gly Thr Ala Asn  
 1155 1160 1165  
 Gly Arg Asn Asp Thr Gly Thr Val Ile Asn Lys Asp Gly Leu Thr Ile  
 1170 1175 1180  
 Thr Leu Ala Asn Gly Ala Ala Ala Gly Thr Asp Ala Ser Asn Gly Asn  
 1185 1190 1195 1200

WO 96/30519

70

Thr Ile Ser Val Thr Lys Asp Gly Ile Ser Ala Gly Asn Lys Glu Ile  
 1205 1210 1215  
 Thr Asn Val Lys Ser Ala Leu Lys Thr Tyr Lys Asp Thr Gln Asn Thr  
 1220 1225 1230  
 Ala Asp Glu Thr Gln Asp Lys Glu Phe His Ala Ala Val Lys Asn Ala  
 1235 1240 1245  
 Asn Glu Val Glu Phe Val Gly Lys Asn Gly Ala Thr Val Ser Ala Lys  
 1250 1255 1260  
 Thr Asp Asn Asn Gly Lys His Thr Val Thr Ile Asp Val Ala Glu Ala  
 1265 1270 1275 1280  
 Lys Val Gly Asp Gly Leu Glu Lys Asp Thr Asp Gly Lys Ile Lys Leu  
 1285 1290 1295  
 Lys Val Asp Asn Thr Asp Gly Asn Asn Leu Leu Thr Val Asp Ala Thr  
 1300 1305 1310  
 Lys Gly Ala Ser Val Ala Lys Gly Glu Phe Asn Ala Val Thr Thr Asp  
 1315 1320 1325  
 Ala Thr Thr Ala Gln Gly Thr Asn Ala Asn Glu Arg Gly Lys Val Val  
 1330 1335 1340  
 Val Lys Gly Ser Asn Gly Ala Thr Ala Thr Glu Thr Asp Lys Lys Lys  
 1345 1350 1355 1360  
 Val Ala Thr Val Gly Asp Val Ala Lys Ala Ile Asn Asp Ala Ala Thr  
 1365 1370 1375  
 Phe Val Lys Val Glu Asn Asp Asp Ser Ala Thr Ile Asp Asp Ser Pro  
 1380 1385 1390  
 Thr Asp Asp Gly Ala Asn Asp Ala Leu Lys Ala Gly Asp Thr Leu Thr  
 1395 1400 1405  
 Leu Lys Ala Gly Lys Asn Leu Lys Val Lys Arg Asp Gly Lys Asn Ile  
 1410 1415 1420  
 Thr Phe Ala Leu Ala Asn Asp Leu Ser Val Lys Ser Ala Thr Val Ser  
 1425 1430 1435 1440  
 Asp Lys Leu Ser Leu Gly Thr Asn Gly Asn Lys Val Asn Ile Thr Ser  
 1445 1450 1455  
 Asp Thr Lys Gly Leu Asn Phe Ala Lys Asp Ser Lys Thr Gly Asp Asp  
 1460 1465 1470  
 Ala Asn Ile His Leu Asn Gly Ile Ala Ser Thr Leu Thr Asp Thr Leu  
 1475 1480 1485  
 Leu Asn Ser Gly Ala Thr Thr Asn Leu Gly Gly Asn Gly Ile Thr Asp  
 1490 1495 1500

Asn Glu Lys Lys Arg Ala Ala Ser Val Lys Asp Val Leu Asn Ala Gly  
 1505 1510 1515 1520  
 Trp Asn Val Arg Gly Val Lys Pro Ala Ser Ala Asn Asn Gln Val Glu  
 1525 1530 1535  
 Asn Ile Asp Phe Val Ala Thr Tyr Asp Thr Val Asp Phe Val Ser Gly  
 1540 1545 1550  
 Asp Lys Asp Thr Thr Ser Val Thr Val Glu Ser Lys Asp Asn Gly Lys  
 1555 1560 1565  
 Arg Thr Glu Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Asp His  
 1570 1575 1580  
 Asn Gly Lys Leu Phe Thr Gly Lys Glu Leu Lys Asp Ala Asn Asn Asn  
 1585 1590 1595 1600  
 Gly Val Thr Val Thr Glu Thr Asp Gly Lys Asp Glu Gly Asn Gly Leu  
 1605 1610 1615  
 Val Thr Ala Lys Ala Val Ile Asp Ala Val Asn Lys Ala Gly Trp Arg  
 1620 1625 1630  
 Val Lys Thr Thr Gly Ala Asn Gly Gln Asn Asp Asp Phe Ala Thr Val  
 1635 1640 1645  
 Ala Ser Gly Thr Asn Val Thr Phe Ala Asp Gly Asn Gly Thr Thr Ala  
 1650 1655 1660  
 Glu Val Thr Lys Ala Asn Asp Gly Ser Ile Thr Val Lys Tyr Asn Val  
 1665 1670 1675 1680  
 Lys Val Ala Asp Gly Leu Lys Leu Asp Gly Asp Lys Ile Val Ala Asp  
 1685 1690 1695  
 Thr Thr Val Leu Thr Val Ala Asp Gly Lys Val Thr Ala Pro Asn Asn  
 1700 1705 1710  
 Gly Asp Gly Lys Lys Phe Val Asp Ala Ser Gly Leu Ala Asp Ala Leu  
 1715 1720 1725  
 Asn Lys Leu Ser Trp Thr Ala Thr Ala Gly Lys Glu Gly Thr Gly Glu  
 1730 1735 1740  
 Val Asp Pro Ala Asn Ser Ala Gly Gln Glu Val Lys Ala Gly Asp Lys  
 1745 1750 1755 1760  
 Val Thr Phe Lys Ala Gly Asp Asn Leu Lys Ile Lys Gln Ser Gly Lys  
 1765 1770 1775  
 Asp Phe Thr Tyr Ser Leu Lys Lys Glu Leu Lys Asp Leu Thr Ser Val  
 1780 1785 1790  
 Glu Phe Lys Asp Ala Asn Gly Gly Thr Gly Ser Glu Ser Thr Lys Ile  
 1795 1800 1805

Thr Lys Asp Gly Leu Thr Ile Thr Pro Ala Asn Gly Ala Gly Ala Ala  
1810 1815 1820

Gly Ala Asn Thr Ala Asn Thr Ile Ser Val Thr Lys Asp Gly Ile Ser  
1825 1830 1835 1840

Ala Gly Asn Lys Ala Val Thr Asn Val Val Ser Gly Leu Lys Lys Phe  
1845 1850 1855

Gly Asp Gly His Thr Leu Ala Asn Gly Thr Val Ala Asp Phe Glu Lys  
1860 1865 1870

His Tyr Asp Asn Ala Tyr Lys Asp Leu Thr Asn Leu Asp Glu Lys Gly  
1875 1880 1885

Ala Asp Asn Asn Pro Thr Val Ala Asp Asn Thr Ala Ala Thr Val Gly  
1890 1895 1900

Asp Leu Arg Gly Leu Gly Trp Val Ile Ser Ala Asp Lys Thr Thr Gly  
1905 1910 1915 1920

Glu Pro Asn Gln Glu Tyr Asn Ala Gln Val Arg Asn Ala Asn Glu Val  
1925 1930 1935

Lys Phe Lys Ser Gly Asn Gly Ile Asn Val Ser Gly Lys Thr Leu Asn  
1940 1945 1950

Gly Thr Arg Val Ile Thr Phe Glu Leu Ala Lys Gly Glu Val Val Lys  
1955 1960 1965

Ser Asn Glu Phe Thr Val Lys Asn Ala Asp Gly Ser Glu Thr Asn Leu  
1970 1975 1980

Val Lys Val Gly Asp Met Tyr Tyr Ser Lys Glu Asp Ile Asp Pro Ala  
1985 1990 1995 2000

Thr Ser Lys Pro Met Thr Gly Lys Thr Glu Lys Tyr Lys Val Glu Asn  
2005 2010 2015

Gly Lys Val Val Ser Ala Asn Gly Ser Lys Thr Glu Val Thr Leu Thr  
2020 2025 2030

Asn Lys Gly Ser Gly Tyr Val Thr Gly Asn Gln Val Ala Asp Ala Ile  
2035 2040 2045

Ala Lys Ser Gly Phe Glu Leu Gly Leu Ala Asp Ala Ala Glu Ala Glu  
2050 2055 2060

Lys Ala Phe Ala Glu Ser Ala Lys Asp Lys Gln Leu Ser Lys Asp Lys  
2065 2070 2075 2080

Ala Glu Thr Val Asn Ala His Asp Lys Val Arg Phe Ala Asn Gly Leu  
2085 2090 2095

Asn Thr Lys Val Ser Ala Ala Thr Val Glu Ser Thr Asp Ala Asn Gly  
2100 2105 2110

73

Asp Lys Val Thr Thr Thr Phe Val Lys Thr Asp Val Glu Leu Pro Leu  
 2115 2120 2125  
 Thr Gln Ile Tyr Asn Thr Asp Ala Asn Gly Asn Lys Ile Val Lys Lys  
 2130 2135 2140  
 Ala Asp Gly Lys Trp Tyr Glu Leu Asn Ala Asp Gly Thr Ala Ser Asn  
 2145 2150 2155 2160  
 Lys Glu Val Thr Leu Gly Asn Val Asp Ala Asn Gly Lys Lys Val Val  
 2165 2170 2175  
 Lys Val Thr Glu Asn Gly Ala Asp Lys Trp Tyr Tyr Thr Asn Ala Asp  
 2180 2185 2190  
 Gly Ala Ala Asp Lys Thr Lys Gly Glu Val Ser Asn Asp Lys Val Ser  
 2195 2200 2205  
 Thr Asp Glu Lys His Val Val Arg Leu Asp Pro Asn Asn Gln Ser Asn  
 2210 2215 2220  
 Gly Lys Gly Val Val Ile Asp Asn Val Ala Asn Gly Glu Ile Ser Ala  
 2225 2230 2235 2240  
 Thr Ser Thr Asp Ala Ile Asn Gly Ser Gln Leu Tyr Ala Val Ala Lys  
 2245 2250 2255  
 Gly Val Thr Asn Leu Ala Gly Gln Val Asn Asn Leu Glu Gly Lys Val  
 2260 2265 2270  
 Asn Lys Val Gly Lys Arg Ala Asp Ala Gly Thr Ala Ser Ala Leu Ala  
 2275 2280 2285  
 Ala Ser Gln Leu Pro Gln Ala Thr Met Pro Gly Lys Ser Met Val Ala  
 2290 2295 2300  
 Ile Ala Gly Ser Ser Tyr Gln Gly Gln Asn Gly Leu Ala Ile Gly Val  
 2305 2310 2315 2320  
 Ser Arg Ile Ser Asp Asn Gly Lys Val Ile Ile Arg Leu Ser Gly Thr  
 2325 2330 2335  
 Thr Asn Ser Gln Gly Lys Thr Gly Val Ala Ala Gly Val Gly Tyr Gln  
 2340 2345 2350  
 Trp

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 658 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

WO 96/30519

74

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Val Thr Gln Thr Trp  
 1 5 10 15

Val Val Val Ser Glu Leu Thr Arg Thr His Thr Lys Cys Ala Ser Ala  
 20 25 30

Thr Val Ala Val Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Glu  
 35 40 45

Ala Asn Asn Asn Thr Pro Val Thr Asn Lys Leu Lys Ala Tyr Gly Asp  
 50 55 60

Ala Asn Phe Asn Phe Thr Asn Asn Ser Ile Ala Asp Ala Glu Lys Gln  
 65 70 75 80

Val Gln Glu Ala Tyr Lys Gly Leu Leu Asn Leu Asn Glu Lys Asn Ala  
 85 90 95

Ser Asp Lys Leu Leu Val Glu Asp Asn Thr Ala Ala Thr Val Gly Asn  
 100 105 110

Leu Arg Lys Leu Gly Trp Val Leu Ser Ser Lys Asn Gly Thr Arg Asn  
 115 120 125

Glu Lys Ser Gln Gln Val Lys His Ala Asp Glu Val Leu Phe Glu Gly  
 130 135 140

Lys Gly Val Gln Val Thr Ser Thr Ser Glu Asn Gly Lys His Thr  
 145 150 155 160

Ile Thr Phe Ala Leu Ala Lys Asp Leu Gly Val Lys Thr Ala Thr Val  
 165 170 175

Ser Asp Thr Leu Thr Ile Gly Gly Ala Ala Ala Gly Ala Thr Thr  
 180 185 190

Thr Pro Lys Val Asn Val Thr Ser Thr Thr Asp Gly Leu Lys Phe Ala  
 195 200 205

Lys Asp Ala Ala Gly Ala Asn Gly Asp Thr Thr Val His Leu Asn Gly  
 210 215 220

Ile Gly Ser Thr Leu Thr Asp Thr Leu Val Gly Ser Pro Ala Thr His  
 225 230 235 240

Ile Asp Gly Gly Asp Gln Ser Thr His Tyr Thr Arg Ala Ala Ser Ile  
 245 250 255

Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Ala Gly  
 260 265 270

Ser Thr Thr Gly Gln Ser Glu Asn Val Asp Phe Val His Thr Tyr Asp  
 275 280 285

Thr Val Glu Phe Leu Ser Ala Asp Thr Glu Thr Thr Val Thr Val  
 290 295 300

Asp Ser Lys Glu Asn Gly Lys Arg Thr Glu Val Lys Ile Gly Ala Lys  
 305 310 315 320

Thr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Ala  
 325 330 335

Asn Lys Glu Thr Asn Lys Val Asp Gly Ala Asn Ala Thr Glu Asp Ala  
 340 345 350

Asp Glu Gly Lys Gly Leu Val Thr Ala Lys Asp Val Ile Asp Ala Val  
 355 360 365

Asn Lys Thr Gly Trp Arg Ile Lys Thr Thr Asp Ala Asn Gly Gln Asn  
 370 375 380

Gly Asp Phe Ala Thr Val Ala Ser Gly Thr Asn Val Thr Phe Ala Ser  
 385 390 395 400

Gly Asn Gly Thr Thr Ala Thr Val Thr Asn Gly Thr Asp Gly Ile Thr  
 405 410 415

Val Lys Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Leu Asp Gly Asp  
 420 425 430

Lys Ile Ala Ala Asp Thr Thr Ala Leu Thr Val Asn Asp Gly Lys Asn  
 435 440 445

Ala Asn Asn Pro Lys Gly Lys Val Ala Asp Val Ala Ser Thr Asp Glu  
 450 455 460

Lys Lys Leu Val Thr Ala Lys Gly Leu Val Thr Ala Leu Asn Ser Leu  
 465 470 475 480

Ser Trp Thr Thr Ala Ala Glu Ala Asp Gly Gly Thr Leu Asp Gly  
 485 490 495

Asn Ala Ser Glu Gln Glu Val Lys Ala Gly Asp Lys Val Thr Phe Lys  
 500 505 510

Ala Gly Lys Asn Leu Lys Val Lys Gln Glu Gly Ala Asn Phe Thr Tyr  
 515 520 525

Ser Leu Gln Asp Ala Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Thr  
 530 535 540

Gly Asn Asn Gly Ala Lys Thr Glu Ile Asn Lys Asp Gly Leu Thr Ile  
 545 550 555 560

Thr Pro Ala Asn Gly Ala Gly Ala Asn Asn Ala Asn Thr Ile Ser Val  
 565 570 575

76

Thr Lys Asp Gly Ile Ser Ala Gly Gly Gln Ser Val Lys Asn Val Val  
 580 585 590  
 Ser Gly Leu Lys Lys Phe Gly Asp Ala Asn Phe Asp Pro Leu Thr Ser  
 595 600 605  
 Ser Ala Asp Asn Leu Thr Lys Gln Asn Asp Asp Ala Tyr Lys Gly Leu  
 610 615 620  
 Thr Asn Leu Asp Glu Lys Gly Thr Asp Lys Gln Thr Pro Val Val Ala  
 625 630 635 640  
 Asp Asn Thr Ala Ala Thr Val Gly Asp Leu Arg Gly Leu Gly Trp Val  
 645 650 655  
 Ile Ser

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 607 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Met Thr Gln Thr Trp  
 1 5 10 15  
 Val Val Val Ser Glu Leu Thr Arg Thr His Thr Lys Arg Leu Arg Asn  
 20 25 30  
 Arg Gly Asp Pro Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln Ala  
 35 40 45  
 Asn Ala Thr Asp Glu Asp Glu Glu Leu Asp Pro Val Val Arg Thr Ala  
 50 55 60  
 Pro Val Leu Ser Phe His Ser Asp Lys Glu Gly Thr Gly Glu Lys Glu  
 65 70 75 80  
 Val Thr Glu Asn Ser Asn Trp Gly Ile Tyr Phe Asp Asn Lys Gly Val  
 85 90 95  
 Leu Lys Ala Gly Ala Ile Thr Leu Lys Ala Gly Asp Asn Leu Lys Xaa  
 100 105 110  
 Lys Gln Xaa Thr Asp Glu Xaa Thr Asn Ala Ser Ser Phe Thr Tyr Ser  
 115 120 125  
 Leu Lys Lys Asp Leu Thr Asp Leu Thr Ser Val Ala Thr Glu Lys Leu  
 130 135 140

Ser Phe Gly Ala Asn Gly Asp Lys Val Asp Ile Thr Ser Asp Ala Asn  
145 150 155 160

Gly Leu Lys Leu Ala Lys Thr Gly Asn Gly Asn Val His Leu Asn Gly  
165 170 175

Leu Asp Ser Thr Leu Pro Asp Ala Val Thr Asn Thr Gly Val Leu Ser  
180 185 190

Ser Ser Ser Phe Thr Pro Asn Asp Val Glu Lys Thr Arg Ala Ala Thr  
195 200 205

Val Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Ala Lys Thr  
210 215 220

Ala Gly Gly Asn Val Glu Ser Val Asp Leu Val Ser Ala Tyr Asn Asn  
225 230 235 240

Val Glu Phe Ile Thr Gly Asp Lys Asn Thr Leu Asp Val Val Leu Thr  
245 250 255

Ala Lys Glu Asn Xaa Lys Thr Thr Glu Val Lys Phe Thr Pro Lys Thr  
260 265 270

Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Glu Asn  
275 280 285

Asn Asp Thr Asn Lys Val Thr Ser Asn Thr Ala Thr Asp Asn Thr Asp  
290 295 300

Glu Gly Asn Gly Leu Val Thr Ala Lys Ala Val Ile Asp Ala Val Asn  
305 310 315 320

Lys Ala Gly Trp Arg Val Lys Thr Thr Ala Asn Gly Gln Asn Gly  
325 330 335

Asp Phe Ala Thr Val Ala Ser Gly Thr Asn Val Thr Phe Glu Ser Gly  
340 345 350

Asp Gly Thr Thr Ala Ser Val Thr Lys Asp Thr Asn Gly Asn Gly Ile  
355 360 365

Thr Val Lys Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Phe Asp Ser  
370 375 380

Asp Lys Lys Ile Val Ala Asp Thr Thr Ala Leu Thr Val Thr Gly Gly  
385 390 395 400

Lys Val Ala Glu Ile Ala Lys Glu Asp Asp Lys Lys Lys Leu Val Asn  
405 410 415

Ala Gly Asp Leu Val Thr Ala Leu Gly Asn Leu Ser Trp Lys Ala Lys  
420 425 430

Ala Glu Ala Asp Thr Asp Gly Ala Leu Glu Gly Ile Ser Lys Asp Gln  
435 440 445

Glu Val Lys Ala Gly Glu Thr Val Thr Phe Lys Ala Gly Lys Asn Leu  
 450 455 460  
 Lys Val Lys Gln Asp Gly Ala Asn Phe Thr Tyr Ser Leu Gln Asp Ala  
 465 470 475 480  
 Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Gly Thr Thr Asn Gly Gly  
 485 490 495  
 Asn Asp Ala Lys Thr Val Ile Asn Lys Asp Gly Leu Thr Ile Thr Pro  
 500 505 510  
 Ala Gly Asn Gly Gly Thr Thr Gly Thr Asn Thr Ile Ser Val Thr Lys  
 515 520 525  
 Asp Gly Ile Lys Ala Gly Asn Lys Ala Ile Thr Asn Val Ala Ser Gly  
 530 535 540  
 Leu Arg Ala Tyr Asp Asp Ala Asn Phe Asp Val Leu Asn Asn Ser Ala  
 545 550 555 560  
 Thr Asp Leu Asn Arg His Val Glu Asp Ala Tyr Lys Gly Leu Leu Asn  
 565 570 575  
 Leu Asn Glu Lys Asn Ala Asn Lys Gln Pro Leu Val Thr Asp Ser Thr  
 580 585 590  
 Ala Ala Thr Val Gly Asp Leu Arg Lys Leu Gly Trp Val Val Ser  
 595 600 605

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Met Thr Gln Thr Trp  
 1 5 10 15  
 Val Val Val Ser Glu Leu Thr Arg  
 20

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Val Thr Gln Thr Trp  
1 5 10 15

Val Val Val Ser Glu Leu Thr Arg  
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu  
1 5 10 15

Val Ala Val Ser Glu Leu Ala Arg  
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu  
1 5 10 15

Val Ala Val Ser Glu Leu Ala Arg  
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Asn Lys Ala Tyr Ser Ile Ile Trp Ser His Ser Arg Gln Ala Trp  
1 5 10 15  
Ile Val Ala Ser Glu Leu Ala Arg  
20

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Asn Arg Ile Tyr Ser Leu Arg Tyr Ser Ala Val Ala Arg Gly Phe  
1 5 10 15  
Ile Ala Val Ser Glu Phe Ala Arg  
20

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Asn Lys Ile Tyr Tyr Leu Lys Tyr Cys His Ile Thr Lys Ser Leu  
1 5 10 15  
Ile Ala Val Ser Glu Leu Ala Arg  
20

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2037 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGAACAAAA	TTTTAACGT	TATTTGAAT	GTTGTGACTC	AAACTGGGT	TGTCGTATCT	60
GAACTCACTC	GCACCCACAC	CAAATGCGCC	TCCGCCACCG	TGGCAGTTGC	CGTATTGGCA	120
ACCCCTGTTGT	CCGCAACGGT	TCAGGCGAAT	GCTACCGATG	AAAACGAAGA	TGATGAAGAA	180
GAGTTAGAAC	CCGTACAACG	CTCTGTTTA	AGGTGGAGCT	TCAAATCCGC	TAAGGAAGGC	240
ACTGGAGAAC	AAGAGGAAAC	AACAGAGGTA	ATAAATTGA	ACACAGATT	ATCAGGAAAT	300
GCAGTAGGAA	GCAGCACAAT	CACCTCAAA	GCCGGCGACA	ACCTGAAAAT	CAAACAAAGC	360
GGCAATGACT	TCACCTACTC	GCTGAAAAAA	GAGCTGAAAAA	ACCTGACCAG	TGTTGAAACT	420
GAAAAATTAT	CGTTGGCGC	AAACGGCAAT	AAAGTTGATA	TTACCAAGTGA	TGCAAATGGC	480
TTGAAATTGG	CGAAAACAGG	TAACGAAAT	GGTCAAAACA	GTAATGTTCA	CTTAAACGGT	540
ATTGCTTCGA	CTTGACCGA	TACGCTTGCC	GGTGGCACAA	CAGGACACGT	TGACACCAAC	600
ATTGATGCGG	TTAATTATCA	TCGCGCTGCA	AGCGTACAAG	ATGTGTTAAA	CAGCGGTTGG	660
AATATCCAAG	GCAATGGAAA	CAATGTCGAT	TTTGTCCGTA	CTTACGACAC	CGTGGACTTT	720
GTCAATGGCG	CGAATGCCAA	TGTGAGCGTT	ACGGCTGATA	CGGCTCACAA	AAAGACAACT	780
GTCCGTGTGG	ATGTAACAGG	CTTGGCCGGTT	CAATATGTTA	CGGAAGACGG	CAAAACCGTT	840
GTGAAAGTGG	GCAATGAGTA	TTACAAAGCC	AAAGATGACG	GTTCGGCGGA	TATGAATCAA	900
AAAGTCGAAA	ACGGCGAGCT	GGCGAAAACC	AAAGTGAAT	TGGTATCGGC	AAGCGGTACA	960
AATCCGGTGA	AAATTAGCAA	TGTTGCAGAC	GGCACGGAAG	ACACCGATGC	GGTCAGCTTT	1020
AAGCAATTAA	AAGCCTTGCA	AGACAAACAG	GTTACGTTGA	GCACGAGCAA	TGCTTATGCC	1080
AATGGCGGTA	CAGATAACGA	CGGCGGCAAG	GCAACTCAA	CTTTAAGCAA	TGGTTTGAAT	1140
TTTAAATTAA	AATCTAGCGA	TGGCGAGTTG	TTGAAAATTA	GCGCGACCGG	CGATACGGTT	1200
ACTTTTACGC	CGAAAAAAAGG	TTCGGTACAG	GTTGGCGATG	ATGGCAAGGC	TTCAATTCA	1260
AAAGGTGCAA	ATACAACGTGA	AGGTTGGTT	GAGGCTTCTG	AATTGGTTGA	AAGCCTGAAC	1320
AAACTGGGTT	GGAAAGTAGG	GGTTGAGAAA	GTCGGCAGCG	GCGAGCTTGA	TGGTACATCC	1380
AAGGAAACTT	TAGTGAAGTC	GGGCGATAAA	GTAACTTGA	AAGCCGGCGA	CAATCTGAAG	1440
GTCAAACAAAG	AGGGCACAAA	CTTCACCTAC	GCGCTCAAAG	ATGAATTGAC	GGCGGTGAAG	1500
AGCGTGGAGT	TTAAAGACAC	GGCGAATGGT	GCAAACGGTG	CAAGCACGAA	GATTACCAA	1560

GACGGCTTGA CCATTACGCT GGCAAACGGT GCGAATGGTG CGACGGTGAC TGATGCCGAC	1620
AAGATTAAG TTGCTTCGGA CGGCATTAGC GCGGGTAATA AAGCAGTTAA AAACGTCGCG	1680
GCAGGCAGAA TTTCTGCCAC TTCCACCGAT GCGATTAACG GAAGCCAGTT GTATGCCGTG	1740
GCAAAAGGGG TAACAAACCT TGCTGGACAA GTGAATAATC TTGAGGGCAA AGTGAATAAA	1800
GTGGGCAAAC GTGCAGATGC AGGTACTGCA AGTGCATTAG CGGCTTCACA GTTACCAACAA	1860
GCCACTATGC CAGGTAAATC AATGGTTCT ATTGCAGGAA GTAGTTATCA AGGTCAAAAT	1920
GGTTTAGCTA TCAGGGTATC AAGAATTCC GATAATGGCA AAGTGATTAT TCGCTTGTCT	1980
GGCACAAACCA ATAGTCAAGG TAAACAGGC GTTGCAGCAG GTGTTGGTTA CCAGTGG	2037

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 679 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Val Thr Gln Thr Trp			
1	5	10	15
Val Val Val Ser Glu Leu Thr Arg Thr His Thr Lys Cys Ala Ser Ala			
20	25	30	
Thr Val Ala Val Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Gln			
35	40	45	
Ala Asn Ala Thr Asp Glu Asn Glu Asp Asp Glu Glu Glu Leu Glu Pro			
50	55	60	
Val Gln Arg Ser Val Leu Arg Trp Ser Phe Lys Ser Ala Lys Glu Gly			
65	70	75	80
Thr Gly Glu Gln Glu Gly Thr Thr Glu Val Ile Asn Leu Asn Thr Asp			
85	90	95	
Ser Ser Gly Asn Ala Val Gly Ser Ser Thr Ile Thr Phe Lys Ala Gly			
100	105	110	
Asp Asn Leu Lys Ile Lys Gln Ser Gly Asn Asp Phe Thr Tyr Ser Leu			
115	120	125	
Lys Lys Glu Leu Lys Asn Leu Thr Ser Val Glu Thr Glu Lys Leu Ser			
130	135	140	

Phe Gly Ala Asn Gly Asn Lys Val Asp Ile Thr Ser Asp Ala Asn Gly  
 145 150 155 160

Leu Lys Leu Ala Lys Thr Gly Asn Gly Gln Asn Ser Asn Val  
 165 170 175

His Leu Asn Gly Ile Ala Ser Thr Leu Thr Asp Thr Leu Ala Gly Gly  
 180 185 190

Thr Thr Gly His Val Asp Thr Asn Ile Asp Ala Val Asn Tyr His Arg  
 195 200 205

Ala Ala Ser Val Gln Asp Val Leu Asn Ser Gly Trp Asn Ile Gln Gly  
 210 215 220

Asn Gly Asn Asn Val Asp Phe Val Arg Thr Tyr Asp Thr Val Asp Phe  
 225 230 235 240

Val Asn Gly Ala Asn Ala Asn Val Ser Val Thr Ala Asp Thr Ala His  
 245 250 255

Lys Lys Thr Thr Val Arg Val Asp Val Thr Gly Leu Pro Val Gln Tyr  
 260 265 270

Val Thr Glu Asp Gly Lys Thr Val Val Lys Val Gly Asn Glu Tyr Tyr  
 275 280 285

Lys Ala Lys Asp Asp Gly Ser Ala Asp Met Asn Gln Lys Val Glu Asn  
 290 295 300

Gly Glu Leu Ala Lys Thr Lys Val Lys Leu Val Ser Ala Ser Gly Thr  
 305 310 315 320

Asn Pro Val Lys Ile Ser Asn Val Ala Asp Gly Thr Glu Asp Thr Asp  
 325 330 335

Ala Val Ser Phe Lys Gln Leu Lys Ala Leu Gln Asp Lys Gln Val Thr  
 340 345 350

Leu Ser Thr Ser Asn Ala Tyr Ala Asn Gly Gly Thr Asp Asn Asp Gly  
 355 360 365

Gly Lys Ala Thr Gln Thr Leu Ser Asn Gly Leu Asn Phe Lys Phe Lys  
 370 375 380

Ser Ser Asp Gly Glu Leu Leu Lys Ile Ser Ala Thr Gly Asp Thr Val  
 385 390 395 400

Thr Phe Thr Pro Lys Lys Gly Ser Val Gln Val Gly Asp Asp Gly Lys  
 405 410 415

Ala Ser Ile Ser Lys Gly Ala Asn Thr Thr Glu Gly Leu Val Glu Ala  
 420 425 430

Ser Glu Leu Val Glu Ser Leu Asn Lys Leu Gly Trp Lys Val Gly Val  
 435 440 445

Glu Lys Val Gly Ser Gly Glu Leu Asp Gly Thr Ser Lys Glu Thr Leu  
450 455 460

Val Lys Ser Gly Asp Lys Val Thr Leu Lys Ala Gly Asp Asn Leu Lys  
465 470 475 480

Val Lys Gln Glu Gly Thr Asn Phe Thr Tyr Ala Leu Lys Asp Glu Leu  
485 490 495

Thr Gly Val Lys Ser Val Glu Phe Lys Asp Thr Ala Asn Gly Ala Asn  
500 505 510

Gly Ala Ser Thr Lys Ile Thr Lys Asp Gly Leu Thr Ile Thr Leu Ala  
515 520 525

Asn Gly Ala Asn Gly Ala Thr Val Thr Asp Ala Asp Lys Ile Lys Val  
530 535 540

Ala Ser Asp Gly Ile Ser Ala Gly Asn Lys Ala Val Lys Asn Val Ala  
545 550 555 560

Ala Gly Glu Ile Ser Ala Thr Ser Thr Asp Ala Ile Asn Gly Ser Gln  
565 570 575

Leu Tyr Ala Val Ala Lys Gly Val Thr Asn Leu Ala Gly Gln Val Asn  
580 585 590

Asn Leu Glu Gly Lys Val Asn Lys Val Gly Lys Arg Ala Asp Ala Gly  
595 600 605

Thr Ala Ser Ala Leu Ala Ala Ser Gln Leu Pro Gln Ala Thr Met Pro  
610 615 620

Gly Lys Ser Met Val Ser Ile Ala Gly Ser Ser Tyr Gln Gly Gln Asn  
625 630 635 640

Gly Leu Ala Ile Gly Val Ser Arg Ile Ser Asp Asn Gly Lys Val Ile  
645 650 655

Ile Arg Leu Ser Gly Thr Thr Asn Ser Gln Gly Lys Thr Gly Val Ala  
660 665 670

Ala Gly Val Gly Tyr Gln Trp  
675

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCGTGCTTGC CCAACACGCT T

21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCTGCCACCT TGCACAAACAA C

21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTTTCAATGC CAGAAAGTAG G

21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTTCAACCGT TGGGGACAAC A

21

## CLAIMS

We claim:

1. A recombinant *Haemophilus* adhesion protein.
2. A recombinant *Haemophilus* adhesion protein according to claim 1 which has  
5 a sequence homologous to that shown in Figure 2.
3. A recombinant *Haemophilus* adhesion protein according to claim 1 which has  
a sequence homologous to the amino acid sequence shown in Figure 3.
4. A recombinant *Haemophilus* adhesion protein according to claim 1 which has  
the sequence shown in Figure 2.
- 10 5. A recombinant *Haemophilus* adhesion protein according to claim 1 which has  
the amino acid sequence shown in Figure 3.
6. A recombinant nucleic acid encoding an *Haemophilus* adhesion protein.
7. The nucleic acid of claim 6 comprising DNA having a sequence homologous to  
that shown in Figure 1.
- 15 8. The nucleic acid of claim 6 comprising DNA having a sequence homologous to  
that shown in Figure 3.
9. The nucleic acid of claim 6 comprising DNA capable of hybridizing to that shown  
in Figure 1.
- 20 10. The nucleic acid of claim 6 comprising DNA capable of hybridizing to that shown  
in Figure 3.

- 87 -

11. The nucleic acid of claim 6 comprising DNA having the sequence shown in Figure 1.
12. The nucleic acid of claim 6 comprising DNA having the sequence shown in Figure 3.
- 5 13. An expression vector comprising transcriptional and translational regulatory nucleic acid operably linked to nucleic acid encoding an *Haemophilus* adhesion protein.
14. A host cell transformed with an expression vector comprising a nucleic acid encoding an *Haemophilus* adhesion protein.
- 10 15. A method of producing an *Haemophilus* adhesion protein comprising:
  - a) culturing a host cell transformed with an expressing vector comprising a nucleic acid encoding an *Haemophilus* adhesion protein; and
  - b) expressing said nucleic acid to produce an *Haemophilus* adhesion protein.
- 15 16. A vaccine comprising a pharmaceutically acceptable carrier and an *Haemophilus* adhesion protein for prophylactic or therapeutic use in generating an immune response.
17. A vaccine according to claim 16 wherein said *Haemophilus* adhesion protein has a sequence homologous to that shown in Figure 2.
- 20 18. A vaccine according to claim 16 wherein said *Haemophilus* adhesion protein has a sequence homologous to the amino acid sequence shown in Figure 3.
19. A monoclonal antibody capable of binding to an *Haemophilus* adhesion protein.

- 88 -

20. A method of treating or preventing *Haemophilus influenzae* infection comprising administering the vaccine of claim 16.

21. A method of treating or preventing a *Haemophilus influenzae* infection according to claim 20 wherein said *H. influenzae* infection is caused by a non-typable *H. influenzae*.

5

ATGAACAAAA	TTTTAACGT	TATTTGGAAT	GTTGTGACTC	AAACTTGGGT	TGTCGTATCT	60
GAACTCACTC	GCACCCACAC	CAAATGCGCC	TCCGCCACCG	TGGCGGTTGC	CGTATTGGCA	120
ACCCCTGTTGT	CCGCAACGGT	TGAGGCGAAC	AAACAATACTC	CTGTTACGAA	TAAGTTGAAG	180
GCTTATGGCG	ATGCGAATT	TAATTCACT	AATAATTGCA	TAGCAGATGC	AGAAAAACAA	240
GTTCAAGAGG	CTTATAAAGG	TTTATTAAAT	CTAAATGAAA	AAAATGCGAG	TGATAAACTG	300
TTGGTGGAGG	ACAATACTGC	GGCGACCGTA	GGCAATTGTC	GTAAATTGGG	CTGGGTATTG	360
TCTAGCAAAA	ACGGCACAAAG	GAACGAGAAA	AGCCAACAAG	TCAAACATGC	GGATGAAAGTG	420
TTGTTGAAG	GCAAAGGCGG	TGTGCAGGTT	ACTTCCACCT	CTGAAAACGG	CAAACACACC	480
ATTACCTTTG	CTTTAGCGAA	AGACCTTGGT	GTGAAAACGT	CGACTGTGAG	TGATACCTTA	540
ACGATTGGCG	GTGGTGCTGC	TGCAGGTGCT	ACAACAACAC	CGAAAGTGAA	TGTAACTAGT	600
ACAACTGATG	GCTTGAAGTT	CGCTAAAGAT	GCTGCGGGTG	CTAATGGCGA	TACTACGGTT	660
CACTTGAATG	GTATTGGTTC	AACCTTGACA	GACACGCTTG	TGGGTTCTCC	TGCTACTCAT	720
ATTGACGGAG	GAGATCAAAG	TACGCATTAC	ACTCGTGCAG	CAAGTATCAA	GGATGTCTTG	780
AATGCGGGTT	GGAATATCAA	GGGTGTTAAA	GCTGGCTCAA	CAACTGGTCA	ATCAGAAAAT	840
GTCGATTTG	TTCATACTTA	CGATACTGTT	GAGTTCTTGA	GTGCGGATAC	AGAGACCACG	900
ACTGTTACTG	TAGATAGCAA	AGAAAACGGT	AAGAGAACCG	AAGTTAAAAT	CGGTGCGAAG	960
ACTTCTGTTA	TCAAAGAAAA	AGACGGTAAG	TTATTTACTG	GAAAAGCTAA	CAAAGAGACA	1020
AATAAAGTTG	ATGGTGCTAA	CGCGACTGAA	GATGCAGACG	AAGGCAAAGG	CTTAGTGACT	1080
GCGAAAGATG	TGATTGACGC	AGTGAATAAG	ACTGGTTGGA	GAATTAAAAC	AACCGATGCT	1140
AATGGTCAA	ATGGCGACTT	CGCAACTGTT	GCATCAGGCA	CAAATGTAAC	CTTGCTAGT	1200
GGTAATGGTA	CAACTGCGAC	TGTAACATAAT	GGCACCGATG	GTATTACCGT	TAAGTATGAT	1260
GCGAAAGTTG	GCGACGGCTT	AAAACTAGAT	GGCGATAAAA	TCGCTGCAGA	TACGACCGCA	1320

**FIG.\_ 1A**



CTTACTGTGA	ATGATGGTAA	GAACGCTAAT	AATCCGAAAG	GTAAAGTGGC	TGATGTTGCT	1380
TCAACTGACG	AGAAGAAATT	GGTTACAGCA	AAAGGTTTAG	TAACAGCCTT	AAACAGTCTA	1440
AGCTGGACTA	CAACTGCTGC	TGAGGGCGGAC	GGTGGTACGC	TTGATGGAAA	TGCAAGTGAG	1500
CAAGAAGTTA	AAGCGGGCGA	AAAGTAACC	TTTAAAGCAG	GCAAGAACCTT	AAAAGTGAAA	1560
CAAGAGGGTG	CGAACTTTAC	TTATTCACTG	CAAGATGCTT	TAACAGGCTT	AACGAGCATT	1620
ACTTTAGGTA	CAGGAATAA	TGGTGCAGAA	ACTGAAATCA	ACAAAGACGG	CTTAACCATC	1680
ACACCAGCAA	ATGGTGCAGG	TGCAAATAAT	GCAAAACACCA	TCAGCGTAAC	CAAAGACGGC	1740
ATTAGTGCAG	GCGGTCAGTC	GGTTAAAAAC	GTTGTGAGCG	GACTGAAGAA	ATTTGGTGAT	1800
GCGAATTTCG	ATCCGCTGAC	TAGCTCCGCC	GACAACCTAA	CGAAACAAAA	TGACGGATGCC	1860
TATAAAGGCT	TGACCAATT	GGATGAAAAA	GGTACAGACA	ACAAACTCC	AGTTGTTGCC	1920
GACAATACCG	CCGCAACCGT	GGGCGATTTG	CGCGGCTTGG	GCTGGGTCA	TTCTGCGGAC	1980
AAAACCACAG	GCGGCTAAC	GGAATATCAC	GATCAAGTTC	GGAATGCGAA	CGAAGTGAAA	2040
TTCAAAAGCG	GCAACGGTAT	CAATGTTCC	GGTAAACGG	TCAACGGTAG	GCGTGAAATT	2100
ACTTTGAAT	TGGCTAAAGG	TGAAGTGGTT	AAATCGAATG	AATTTACCGT	CAAAGAAACC	2160
AATGGAAAGG	AAACGAGCCT	GGTAAAGTT	GGCGATAAAAT	ATTACAGCAA	AGAGGATATT	2220
GAECTAACAA	CAGGTCAGCC	TAAATTAAAA	GATGGCAATA	CAGTTGCTGC	GAAATATCAA	2280
GATAAAGGTG	GCAAAAGTCGT	TTCTGTAACG	GATAATACTG	AAGCTACCAT	ACCAACAAA	2340
GGTTCTGGCT	ATGTAACAGG	TAACCAAGTG	GCAGATGCGA	TTGCGAAATC	AGGCTTGAG	2400
CTTGGCTTGG	CTGATGAAGC	TGATGCGAAA	CGGGCGTTTG	ATGATAAGAC	AAAAGCCTTA	2460
TCTGCTGGTA	CAACGGAAAT	TGTAAATGCC	CACGATAAAAG	TCCGTTTG	TAATGGTTA	2520
AATACCAAAG	TGAGCGCGGC	AACGGTGGAA	AGCACCGATG	CAAACGGCGA	TAAAGTGACC	2580
ACAACCTTG	TGAAAACCGA	TGTGGAATTG	CCTTTAACGC	AAATCTACAA	TACCGATGCA	2640

**FIG.\_1B**

AACGGTAAGA AAATCACTAA AGTTGTCAAA GATGGGCAAA CTAATGGTA TGAACGTGAAT	2700
GCTGACGGTA CGGCTGATAT GACCAAAGAA GTTACCCCTCG GTAACGTGGA TTCAGACGGC	2760
AAGAAAAGTTG TGAAAGACAA CGATGGCAAG TGGTATCACG CCAAAGCTGA CGGTACTGCG	2820
GATAAAACCA AAGGCGAAGT GAGCAATGAT AAAGTTCTA CCGATGAAAA ACACGTTGTC	2880
AGCCTTGATC CAAATGATCA ATCAAAAGGT AAAGGTGTCG TGATTGACAA TGTGGCTAAT	2940
GGCGATATTT CTGCCACTTC CACCGATGCG ATTAACGGAA GTCAGTTGTA TGCTGTGGCA	3000
AAAGGGGTAA CAAACCTTGC TGGACAAGTG AATAATCTTG AGGGCAAAGT GAATAAAGTG	3060
GGCAAACGTG CAGATGCAGG TACAGCAAGT GCATTAGCGG CTTCACAGTT ACCACAAGCC	3120
ACTATGCCAG GTAAATCAAT GGTTGCTATT GCGGGAAGTA GTTATCAAGG TCAAAATGGT	3180
TTAGCTATCG GGGTATCAAG AATTTCGAT AATGGCAAAG TGATTATTG CTTGTCAGGC	3240
ACAACCAATA GTCAAGGTAA AACAGGCGTT GCAGCAGGTG TTGGTTACCA GTGG	3294

## FIG.\_1C

4 / 26

Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Val Thr Gln Thr Trp  
 1 5 10 15

Val Val Val Ser Glu Leu Thr Arg Thr His Thr Lys Cys Ala Ser Ala  
 20 25 30

Thr Val Ala Val Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Glu  
 35 40 45

Ala Asn Asn Asn Thr Pro Val Thr Asn Lys Leu Lys Ala Tyr Gly Asp  
 50 55 60

Ala Asn Phe Asn Phe Thr Asn Asn Ser Ile Ala Asp Ala Glu Lys Gln  
 65 70 75 80

Val Gln Glu Ala Tyr Lys Gly Leu Leu Asn Leu Asn Glu Lys Asn Ala  
 85 90 95

Ser Asp Lys Leu Leu Val Glu Asp Asn Thr Ala Ala Thr Val Gly Asn  
 100 105 110

Leu Arg Lys Leu Gly Trp Val Leu Ser Ser Lys Asn Gly Thr Arg Asn  
 115 120 125

Glu Lys Ser Gln Gln Val Lys His Ala Asp Glu Val Leu Phe Glu Gly  
 130 135 140

Lys Gly Gly Val Gln Val Thr Ser Thr Ser Glu Asn Gly Lys His Thr  
 145 150 155 160

Ile Thr Phe Ala Leu Ala Lys Asp Leu Gly Val Lys Thr Ala Thr Val  
 165 170 175

Ser Asp Thr Leu Thr Ile Gly Gly Ala Ala Ala Gly Ala Thr Thr  
 180 185 190

Thr Pro Lys Val Asn Val Thr Ser Thr Thr Asp Gly Leu Lys Phe Ala  
 195 200 205

Lys Asp Ala Ala Gly Ala Asn Gly Asp Thr Thr Val His Leu Asn Gly  
 210 215 220

Ile Gly Ser Thr Leu Thr Asp Thr Leu Val Gly Ser Pro Ala Thr His  
 225 230 235 240

Ile Asp Gly Gly Asp Gln Ser Thr His Tyr Thr Arg Ala Ala Ser Ile  
 245 250 255

Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Ala Gly  
 260 265 270

Ser Thr Thr Gly Gln Ser Glu Asn Val Asp Phe Val His Thr Tyr Asp  
 275 280 285

5 / 26

Thr Val Glu Phe Leu Ser Ala Asp Thr Glu Thr Thr Thr Val Thr Val  
 290 295 300

Asp Ser Lys Glu Asn Gly Lys Arg Thr Glu Val Lys Ile Gly Ala Lys  
 305 310 315 320

Thr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Ala  
 325 330 335

Asn Lys Glu Thr Asn Lys Val Asp Gly Ala Asn Ala Thr Glu Asp Ala  
 340 345 350

Asp Glu Gly Lys Gly Leu Val Thr Ala Lys Asp Val Ile Asp Ala Val  
 355 360 365

Asn Lys Thr Gly Trp Arg Ile Lys Thr Thr Asp Ala Asn Gly Gln Asn  
 370 375 380

Gly Asp Phe Ala Thr Val Ala Ser Gly Thr Asn Val Thr Phe Ala Ser  
 385 390 395 400

Gly Asn Gly Thr Thr Ala Thr Val Thr Asn Gly Thr Asp Gly Ile Thr  
 405 410 415

Val Lys Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Leu Asp Gly Asp  
 420 425 430

Lys Ile Ala Ala Asp Thr Thr Ala Leu Thr Val Asn Asp Gly Lys Asn  
 435 440 445

Ala Asn Asn Pro Lys Gly Lys Val Ala Asp Val Ala Ser Thr Asp Glu  
 450 455 460

Lys Lys Leu Val Thr Ala Lys Gly Leu Val Thr Ala Leu Asn Ser Leu  
 465 470 475 480

Ser Trp Thr Thr Ala Ala Glu Ala Asp Gly Gly Thr Leu Asp Gly  
 485 490 495

Asn Ala Ser Glu Gln Glu Val Lys Ala Gly Asp Lys Val Thr Phe Lys  
 500 505 510

Ala Gly Lys Asn Leu Lys Val Lys Gln Glu Gly Ala Asn Phe Thr Tyr  
 515 520 525

Ser Leu Gln Asp Ala Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Thr  
 530 535 540

Gly Asn Asn Gly Ala Lys Thr Glu Ile Asn Lys Asp Gly Leu Thr Ile  
 545 550 555 560

Thr Pro Ala Asn Gly Ala Gly Ala Asn Asn Ala Asn Thr Ile Ser Val  
 565 570 575

Thr Lys Asp Gly Ile Ser Ala Gly Gly Gln Ser Val Lys Asn Val Val  
 580 585 590

**FIG.\_2B**

6 / 26

Ser Gly Leu Lys Lys Phe Gly Asp Ala Asn Phe Asp Pro Leu Thr Ser  
 595 600 605

Ser Ala Asp Asn Leu Thr Lys Gln Asn Asp Asp Ala Tyr Lys Gly Leu  
 610 615 620

Thr Asn Leu Asp Glu Lys Gly Thr Asp Lys Gln Thr Pro Val Val Ala  
 625 630 635 640

Asp Asn Thr Ala Ala Thr Val Gly Asp Leu Arg Gly Leu Gly Trp Val  
 645 650 655

Ile Ser Ala Asp Lys Thr Thr Gly Gly Ser Thr Glu Tyr His Asp Gln  
 660 665 670

Val Arg Asn Ala Asn Glu Val Lys Phe Lys Ser Gly Asn Gly Ile Asn  
 675 680 685

Val Ser Gly Lys Thr Val Asn Gly Arg Arg Glu Ile Thr Phe Glu Leu  
 690 695 700

Ala Lys Gly Glu Val Val Lys Ser Asn Glu Phe Thr Val Lys Glu Thr  
 705 710 715 720

Asn Gly Lys Glu Thr Ser Leu Val Lys Val Gly Asp Lys Tyr Tyr Ser  
 725 730 735

Lys Glu Asp Ile Asp Leu Thr Thr Gly Gln Pro Lys Leu Lys Asp Gly  
 740 745 750

Asn Thr Val Ala Ala Lys Tyr Gln Asp Lys Gly Gly Lys Val Val Ser  
 755 760 765

Val Thr Asp Asn Thr Glu Ala Thr Ile Thr Asn Lys Gly Ser Gly Tyr  
 770 775 780

Val Thr Gly Asn Gln Val Ala Asp Ala Ile Ala Lys Ser Gly Phe Glu  
 785 790 795 800

Leu Gly Leu Ala Asp Glu Ala Asp Ala Lys Arg Ala Phe Asp Asp Lys  
 805 810 815

Thr Lys Ala Leu Ser Ala Gly Thr Thr Glu Ile Val Asn Ala His Asp  
 820 825 830

Lys Val Arg Phe Ala Asn Gly Leu Asn Thr Lys Val Ser Ala Ala Thr  
 835 840 845

Val Glu Ser Thr Asp Ala Asn Gly Asp Lys Val Thr Thr Phe Val  
 850 855 860

Lys Thr Asp Val Glu Leu Pro Leu Thr Gln Ile Tyr Asn Thr Asp Ala  
 865 870 875 880

Asn Gly Lys Lys Ile Thr Lys Val Val Lys Asp Gly Gln Thr Lys Trp  
 885 890 895

**FIG.\_2C**

7 / 26

Tyr Glu Leu Asn Ala Asp Gly Thr Ala Asp Met Thr Lys Glu Val Thr  
900 905 910

Leu Gly Asn Val Asp Ser Asp Gly Lys Lys Val Val Lys Asp Asn Asp  
915 920 925

Gly Lys Trp Tyr His Ala Lys Ala Asp Gly Thr Ala Asp Lys Thr Lys  
930 935 940

Gly Glu Val Ser Asn Asp Lys Val Ser Thr Asp Glu Lys His Val Val  
945 950 955 960

Ser Leu Asp Pro Asn Asp Gln Ser Lys Gly Lys Gly Val Val Ile Asp  
965 970 975

Asn Val Ala Asn Gly Asp Ile Ser Ala Thr Ser Thr Asp Ala Ile Asn  
980 985 990

Gly Ser Gln Leu Tyr Ala Val Ala Lys Gly Val Thr Asn Leu Ala Gly  
995 1000 1005

Gln Val Asn Asn Leu Glu Gly Lys Val Asn Lys Val Gly Lys Arg Ala  
1010 1015 1020

Asp Ala Gly Thr Ala Ser Ala Leu Ala Ala Ser Gln Leu Pro Gln Ala  
1025 1030 1035 1040

Thr Met Pro Gly Lys Ser Met Val Ala Ile Ala Gly Ser Ser Tyr Gln  
1045 1050 1055

Gly Gln Asn Gly Leu Ala Ile Gly Val Ser Arg Ile Ser Asp Asn Gly  
1060 1065 1070

Lys Val Ile Ile Arg Leu Ser Gly Thr Thr Asn Ser Gln Gly Lys Thr  
1075 1080 1085

Gly Val Ala Ala Gly Val Gly Tyr Gln Trp  
1090 1095

**FIG.\_2D**

8 / 26

1	TTTNTTTTCTTATTTTTTTTTTTTTTTTGAGGCTAAACTTTNGNA	60
61	AAATATCACTTTTATTCTCAAATATAGAATAGAACGACGATTCACTAAGAAAA	120
121	GTATATTATCATTAAATTATTAAATATA <u>AGGTAA</u> ATAAAATGAACAAAATTTAAC M N K I F N	180
181	GTTATTTGGAATGTTATGACTCAAACCTGGGTGTCGTATCTGAACACTCGCACCCAC V I W N V M T Q T W V V V S E L T R T H	240
241	ACCAAACGCGCCTCCGCAACCGTGGAGACCGCCGTATTGGCGACACTGTTGTTGCAACG T K R A S A T V E T A V L A T L L F A T	300
301	GTTCAGGCCAATGCTACCGATGAAGATGAAGAGTTAGACCCCGTAGTACCGACTGCTCCC V Q A N A T D E D E E L D P V V R T A P	360
361	GTGTTGAGCTTCCATTCCGATAAAAGAAGGCACGGGAGAAAAAGAAGTTACAGAAAATTCA V L S F H S D K E G T G E K E V T E N S	420
421	AATTGGGAATATATTCGACAATAAAGGAGTACTAAAAGCCGGAGCAATCACCCCTCAA N W G I Y F D N K G V L K A G A I T L K	480
481	GCCGGCGACAACCTGAAAATCAAACAAAACACCGATGAAAGCACCAATGCCAGTAGCTTC A G D N L K I K Q N T D E S T N A S S F	540
541	ACCTACTCGCTGAAAAAGACCTCACAGATCTGACCAGTGTGCAACTGAAAATTATCG T Y S L K K D L T D L T S V A T E K L S	600
601	TTTGGCCAAACGGCGATAAAGTTGATATTACCAAGTGATGCAAATGGCTGAAATTGGCG F G A N G D K V D I T S D A N G L K L A	660
661	AAAACAGGTAAACGGAAATGTTCATTTGAATGGTTGGATTCAACTTGCGCTGATGCGGTA K T G N G N V H L N G L D S T L P D A V	720
721	ACGAATACAGGTGTGTTAAGTTCATCAAGTTTACACCTAATGATGTTGAAAAAACAGA T N T G V L S S S F T P N D V E K T R	780
781	GCTGCAACTGTTAAAGATGTTTAAATGCAGGTTGGAACATTAAGGTGCTAAACTGCT A A T V K D V L N A G W N I K G A K T A	840
841	GGAGGTAATGTTGAGAGTGTGATTAGTGTCCGCTTATAATAATGTTGAAATTATTACA G G N V E S V D L V S A Y N N V E F I T	900
901	GGCGATAAAAACACGCTTGATGTTGATTAACAGCTAAAGAAAACGGTAAAACAACCGAA G D K N T L D V V L T A K E N G K T T E	960
961	GTGAAATTCACACCGAAAACCTCTGTTATCAAAGAAAAGACGGTAAGTATTTACTGGA V K F T P K T S V I K E R D G K L F T G	1020
1021	AAAGAGAATAACGACACAAATAAAGTTACAAGTAACACGGCGACTGATAATACAGATGAG K E N N D T N K V T S N T A T D N T D E	1080
1081	GGTAATGGCTTAGTCACTGCAAAAGCTGTGATTGATGCTGTGAACAAGGCTGGTTGGAGA G N G L V T A K A V I D A V N K A G W R	1140

9 / 26

1141	GTTAAAACAAC TACTGCTAATGGTCAAAATGGCGACTTCGCAACTGTTGCGTCAGGCACA V K T T T A N G Q N G D F A T V A S G T	1200
1201	AATGTAACCTTGAAAGTGGCGATGGTACAACAGCGTCAGTAAC TAAAGATACTAACGGC N V T F E S G D G T T A S V T K D T N G	1260
1261	AATGGCATCACTGTTAAGTACGACGCGAAAGTTGGCGACGGCTTGAAATTGATAGCGAT N G I T V K Y D A K V G D G L K F D S D	1320
1321	AAAAAAATCGTGCAGATA CGACCGCACTTACTGTGACAGGTGGTAAGGTAGCTGAAATT K K I V A D T T A L T V T G G K V A E I	1380
1381	GCTAAAGAAGATGACAAGAAAAACTTGTATGCAGGCATTGGTAACAGCTTAGGT A K E D D K K L V N A G D L V T A L G	1440
1441	AATCTAAGTGGAAAGCAAAGCTGAGGCTGATACTGATGGTGCCTTGAGGGGATTCA N L S W K A K A E A D T D G A L E G I S	1500
1501	AAAGACCAAGAAGTCAAAGCAGGCACCGTAACCTTAAAGCGGGCAAGAACTTAAA K D Q E V K A G E T V T F K A G K N L K	1560
1561	GTGAAACAGGATGGTGCACCTTACTTATTCACTGCAAGATGCTTAA CGGGTTAACG V K Q D G A N F T Y S L Q D A L T G L T	1620
1621	AGCATTACTTTAGGTGGTACAACTAATGGCGGAATGATGCGAAAACCGTCATCAACAAA S I T L G G T T N G G N D A K T V I N K	1680
1681	GACGGTTAACCATCACGCCAGCAGGTAA TGGCGGTACGACAGGTACAAACACCATCAGC D G L T I T P A G N G G T T G T N T I S	1740
1741	GTAACCAAAGATGGCATTAAAGCAGGTAA TAAAGCTATTACTAATGTTGCGAGTGGTTA V T K D G I K A G N K A I T N V A S G L	1800
1801	AGAGCTTATGACGATGCGAATTGATGTTAAATAACTCTGCAACTGATTAAATAGA R A Y D D A N F D V L N N S A T D L N R	1860
1861	CACGTTGAAGATGCTTATAAAGGTTATTAAATCTAAATGAAAAAAATGCAAATAACAA H V E D A Y K G L L N L N E K N A N K Q	1920
1921	CCGTTGGTGA CAGCACGGCGACTGTAGGCGATTACGTAAATTGGGTTGGTA P L V T D S T A A T V G D L R K L G W V	1980
1981	GTATCAACCAAAACGGTACGAAAGAAGAAAGCAATCAAGTAAACAAGCTGATGAAGTC V S T K N G T K E E S N Q V K Q A D E V	2040
2041	CTCTTACCGGAGCCGGTGCCTGCTACGGTTACTTCCAAATCTGAAAACGGTAAACATACG L F T G A G A A T V T S K S E N G K H T	2100
2101	ATTACCGTTAGTGTGGCTGAAACTAAAGCGGATTGCGGTCTTGGAAAAAGATGGCATACT I T V S V A E T K A D C G L E K D G D T	2160
2161	ATTAAGCTCAAAGTGGATAATCAAACACTGATAATGTTAACTGTTGGTAATAATGGT I K L K V D N Q N T D N V L T V G N N G	2220
2221	ACTGCTGTCACTAAAGGTGGCTTGAAACTGTTAAA ACTGGAGCGACTGATGCAGATCGC T A V T K G G F E T V K T G A T D A D R	2280

10 / 26

2281	GGTAAAGTAACGTAAAAGATGCTACTGCTAATGACGCTGATAAGAAAGTCGCAACTGTA	2340
	G K V T V K D A T A N D A D K K V A T V	
2341	AAAGATGTTGCAACCGCAATTAAATAGTGCAGCGACTTTGTGAAAACAGAGAATTAACT	2400
	K D V A T A I N S A A T F V K T E N L T	
2401	ACCTCTATTGATGAAGATAATCCTACAGATAACGGCAAAGATGACGCACCTAAAGCGGGC	2460
	T S I D E D N P T D N G K D D A L K A G	
2461	GATACCTAACCTTAAAGCAGGTAAAAACCTGAAAGTTAACGTGATGGAAAAATATT	2520
	D T L T F K A G K N L K V R D G K N I	
2521	ACTTTGACTTGGCGAAAAACCTTGAGGTGAAAAGTGCAGCGACTTGATGAACTTAAACG	2580
	T F D L A K N L E V K T A K V S D T L T	
2581	ATTGGCGGGAAATACACCTACAGGTGGCACTACTGGCACGCCAAAAGTGAATATTACTAGC	2640
	I G G N T P T G G T T A T P K V N I T S	
2641	ACGGCTGATGGTTGAATTTCGAAAGAAAACAGCCGATGCCCTGGGTCTAAGAATGTT	2700
	T A D G L N F A K E T A D A S G S K N V	
2701	TATTTGAAAGGTATTGCGACAACCTTAACTGAGCCAAGCGCGGGAGCGAAGTCTCACAC	2760
	Y L K G I A T T L T E P S A G A K S S H	
2761	GTTGATTAAATGTGGATGCGACGAAAAATCCAATGCAGCAAGTATTGAAGATGTATTG	2820
	V D L N V D A T K K S N A A S I E D V L	
2821	CGCGCAGGTTGGAATTCAAGGTAATGGTAATAATGTTGATTATGTAGCGACGTATGAC	2880
	R A G W N I Q G N G N N V D Y V A T Y D	
2881	ACAGTAAACTTACCGATGACAGCACAGGTACAACAACGTAACCGTAACCCAAAAGCA	2940
	T V N F T D D S T G T T T V T V T Q K A	
2941	GATGGCAAAGGTGCTGACGTTAAATCGGTGCGAAAACCTCTGTTATCAAAGACCACAA	3000
	D G K G A D V K I G A K T S V I K D H N	
3001	GGCAAACGTACAGGCAAAGACCTGAAAGATGCGAATAATGGTCAACCGTTAGTGAA	3060
	G K L F T G K D L K D A N N G A T V S E	
3061	GATGATGGCAAAGACACCGGCACAGGCTTAGTTACTGCAAAACTGTGATTGATGCAGTA	3120
	D D G K D T G T G L V T A K T V I D A V	
3121	AATAAAAGCGGTTGGAGGGTAACCGGTGAGGGCGCAGTGCCTAAACCGGTGCAACCGCC	3180
	N K S G W R V T G E G A T A E T G A T A	
3181	GTAATGCGGGTAACGCTGAAACCGTTACATCAGGCACGAGCGTGAACCTCAAAACGGC	3240
	V N A G N A E T V T S G T S V N F K N G	
3241	AATGCGACCAACAGCGACCGTAAGCAAAGATAATGCCAACATCAATGTCAAATACGATGTA	3300
	N A T T A T V S K D N G N I N V K Y D V	
3301	AATGTTGGTGACGGCTTGAAGATTGGCGATGACAAAAAAATCGTTGCAGACACGACCACA	3360
	N V G D G L K I G D D D K K I V A D T T T	
3361	CTTACTGTAACAGGTGGTAAGGTGTCTGTTCTGCTGGTGCTAATAGTGTAAATAACAAT	3420
	L T V T G G K V S V P A G A N S V N N N	

11 / 26

3421	AAGAAACTTGTAAATGCAGAGGGTTAGCGACTGCTTAAACAACCTAACGCTGGACGGCA K K L V N A E G L A T A L N N L S W T A	3480
3481	AAAGCCGATAAAATATGCAGATGGCGAGTCAGAGGGCGAAACCGACCAAGAAGTCAAAGCA K A D K Y A D G E S E G E T D Q E V K A	3540
3541	GGCGACAAAGTAACCTTAAAGCAGGCAAGAACCTAAAAGTAAAAGCTGAAACAGTCTGAAAAAGAC G D K V T F K A G K N L K V K Q S E K D	3600
3601	TTTACTTATTCACTGCAAGACACTTTAACAGGCTTAACGAGCATTACTTAGGTGGTACA F T Y S L Q D T L T G L T S I T L G G T	3660
3661	GCTAAATGGCAGAAATGATAACGGGAACCGTCATCAACAAAGACGGCTAACCATCACGCTG A N G R N D T G T V I N K D G L T I T L	3720
3721	GCAAATGGTGCTCGGCAGGCACAGATGCGTCTAACGGAAACACCATCAGTGTAAACAAA A N G A A A G T D A S N G N T I S V T K	3780
3781	GACGGCATTAGTGCAGGTAATAAGAAATTACCAATGTTAAGAGTGCTTAAAACCTAT D G I S A G N K E I T N V K S A L K T Y	3840
3841	AAAGATACTCAAAACACTGCAGATGAAACACAAGATAAAAGAGTCCACGCCGCCGTTAAA K D T Q N T A D E T Q D K E F H A A V K	3900
3901	AACGCAAATGAAGTTGAGTCGTGGTAAAAACGGTGCAACCGTGTCTGCAAAACTGAT N A N E V E F V G K N G A T V S A K T D	3960
3961	AACAACGGAAAACATACTGTAACGATTGATGTTGCAGAAGCCAAAGTGGTGTGGTCTT N N G K H T V T I D V A E A K V G D G L	4020
4021	GAAAAAGATACTGACGGCAAGATTAACACTCAAAGTAGATAATAACAGATGGGATAATCTA E K D T D G K I K L K V D N T D G N N L	4080
4081	TTAACCGTTGATGCAACAAAGGTGCATCCGTTGCCAAGGGCGAGTTAACCGCTAAC L T V D A T K G A S V A K G E F N A V T	4140
4141	ACAGATGCAACTACAGCCCAAGGCACAAATGCCAATGAGCGCGGTAAAGTGGTGTCAAG T D A T T A Q G T N A N E R G K V V V K	4200
4201	GGTTCAAATGGTGCACACTGCTACCGAAACTGACAAGAAAAAGTGGCAACTGTTGGCGAC G S N G A T A T E T D K K K V A T V G D	4260
4261	GTTGCTAAAGCGATTAACGACGCAGCAACTTGTGAAAGTGGAAAATGACGACAGTGCT V A K A I N D A A T F V K V E N D D S A	4320
4321	ACGATTGATGATGCCAACAGATGATGGCGCAAATGATGCTCTCAAAGCAGGGCGACACC T I D D S P T D D G A N D A L K A G D T	4380
4381	TTGACCTTAAAGCGGGTAAAAACTTAAAGTAAACGTGATGGTAAAAATATTACTTT L T L K A G K N L K V K R D G K N I T F	4440
4441	GCCCTTGCACGACCTTAGTGTAAAAAGCGCAACCGTTAGCGATAAAATTATCGCTTGGT A L A N D L S V K S A T V S D K L S L G	4500
4501	ACAAACGGCAATAAGTCAATATCACAAGCGACACCAAGGCTTGAACCTCGCTAAAGAT T N G N K V N I T S D T K G L N F A K D	4560

12 / 26

4561	AGTAAGACAGGCATGATGCTAATATTCACTTAAATGGCATTGCTCAACTTAACTGAT	4620
	S K T G D D A N I H L N G I A S T L T D	
4621	ACATTGTTAAATAGTGGTGCAGACAACCAATTAGGTGGTAATGGTATTACTGATAACGAG	4680
	T L L N S G A T T N L G G N G I T D N E	
4681	AAAAAACGCGCGGCGAGCGTTAAAGATGTCTTGAATGCGGGTTGGAATGTCGTGGTGT	4740
	K K R A A S V K D V L N A G W N V R G V	
4741	AAACCGGCATCTGCAAATAATCAAGTGGAGAATATCGACTTGTAGCAACCTACGACACA	4800
	K P A S A N N Q V E N I D F V A T Y D T	
4801	GTGGACTTTGTTAGTGGAGATAAAGACACCACCGAGTGTAACTGTTGAAAGTAAAGATAAT	4860
	V D F V S G D K D T T S V T V E S K D N	
4861	GGCAAGAGAACCGAAGTAAAATCGGTGCGAAGACTTCTGTTATCAAAGACCACACGGC	4920
	G K R T E V K I G A K T S V I K D H N G	
4921	AAACTGTTACAGGCAGAGCTGAAGGATGCTAACATAATGGCGTAACGTGTACCGAA	4980
	K L F T G K E L K D A N N N G V T V T E	
4981	ACCGACGGCAAAGACGAGGGTAATGGTTAGTGACTGCAAAAGCTGTGATTGATGCCGTG	5040
	T D G K D E G N G L V T A K A V I D A V	
5041	AATAAGGCTGGTGGAGAGTTAAAACACAGGTGCTAATGGTCAGAATGATGACTTCGCA	5100
	N K A G W R V K T T G A N G Q N D D F A	
5101	ACTGTTGCGTCAGGCACAAATGTAACCTTGCTGATGGTAATGGCACAACTGCCGAAGTA	5160
	T V A S G T N V T F A D G N G T T A E V	
5161	ACTAAAGCAAACGACGGTAGTATTACTGTTAAATACAATGTTAAAGTGGCTGATGGCTTA	5220
	T K A N D G S I T V K Y N V K V A D G L	
5221	AAACTAGACGGCGATAAAATCGTTGCAGACACGACCGTACTTACTGTGGCAGATGGTAA	5280
	K L D G D K I V A D T T V L T V A D G K	
5281	GTTACAGCTCCGAATAATGGCGATGGTAAGAAATTGTTGATGCAAGTGGTTAGCGGAT	5340
	V T A P N N G D G K K F V D A S G L A D	
5341	GCGTTAAATAATTAAAGCTGGACGGCAACTGCTGGTAAAGAAGGCACGGTGAAGTTGAT	5400
	A L N K L S W T A T A G K E G T G E V D	
5401	CCTGCAAATTCAAGCAGGGCAAGAAGTCAAAGCGGGCGACAAAGTAACCTTAAAGCCGGC	5460
	P A N S A G Q E V K A G D K V T F K A G	
5461	GACAACCTGAAAATCAAACAAAGCGGCAAAGACTTACCTACTCGCTGAAAAAGAGCTG	5520
	D N L K I K Q S G K D F T Y S L K K E L	
5521	AAAGACCTGACCAGCGTAGAGTTCAAAGACGCAAACGGCGGTACAGGCAGTGAAAGCACC	5580
	K D L T S V E F K D A N G G T G S E S T	
5581	AAGATTACCAAAGACGGCTTGACCATTACGCCGGCAAACGGTGCAGGTGCGGCAGGTGCA	5640
	K I T K D G L T I T P A N G A G A A G A	
5641	AACACTGCAAACACCATTAGCGTAACCAAAGATGGCATTAGCGCGGGTAATAAGCAGTT	5700
	N T A N T I S V T K D G I S A G N K A V	

13 / 26

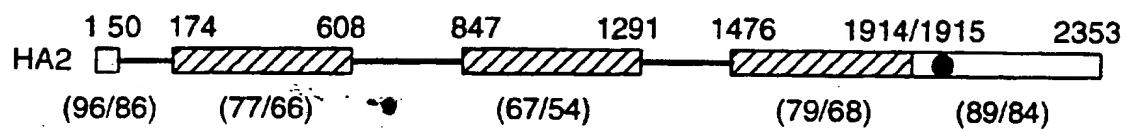
5701	ACAAAACGTTGTGAGCGGACTGAAGAAATTGGTATGGTCATACGTGGCAAATGGCACT T N V V S G L K K F G D G H T L A N G T	5760
5761	GTTGCTGATTTGAAAAGCATTATGACAATGCCTATAAAAGACTTGACCAATTGGATGAA V A D F E K H Y D N A Y K D L T N L D E	5820
5821	AAAGGC CGCGATAATAATCCGACTGTTGCCGACAATACCGCTGCAACCGTGGCGATTG K G A D N N P T V A D N T A A T V G D L	5880
5881	CGCGGCTTGGCTGGTCATTTCTCGGGACAAAACCACAGGCGAACCAATCAGGAATAC R G L G W V I S A D K T T G E P N Q E Y	5940
5941	AACCGCGCAAGTGCCTAACGCCAATGAAGTGAATTCAAGAGCGGCAACGGTATCAATGTT N A Q V R N A N E V K F K S G N G I N V	6000
6001	TCCGGTAAAACATTGAACGGTACGCCGTGATTACCTTGAATTGGCTAAAGGCGAAGTG S G K T L N G T R V I T F E L A K G E V	6060
6061	GTTAAATCGAATGAATTACCGTTAAGAATGCCATGGTCGGAAACGAACTTGGTTAAA V K S N E F T V K N A D G S E T N L V K	6120
6121	GTTGGCGATATGTATTACAGCAAAGAGGGATATTGACCCGGCAACCAGTAAACCGATGACA V G D M Y Y S K E D I D P A T S K P M T	6180
6181	GGTAAAACGTAAAAATATAAGGTTGAAAACGGCAAAGTCGTTCTGCTAACGGCAGCAAG G K T E K Y K V E N G K V V S A N G S K	6240
6241	ACCGAAGTTACCCCTAACCAACAAAGGTTCCGGCTATGTAACAGGTAAACCAAGTGGCTGAT T E V T L T N K G S G Y V T G N Q V A D	6300
6301	GCGATTGCGAAATCAGGCTTGAGCTTGGTTGGCTGATGCCGAGAAGCTGAAAAAGCC A I A K S G F E L G L A D A A E A E K A	6360
6361	TTTGCAGAAAGCGCAAAAGACAAGCAATTGCTAAAGATAAAGCGGAAACTGTAAATGCC F A E S A K D K Q L S K D K A E T V N A	6420
6421	CACGATAAAAGTCCGTTTGCTAATGGTTAAATACCAAAGTGAGCGCGAACGGTGAA H D K V R F A N G L N T K V S A A T V E	6480
6481	AGCACTGATGCAAACGGCATAAAAGTGAACCAACCTTGATGAAAACCGATGTGAAATTG S T D A N G D K V T T T F V K T D V E L	6540
6541	CCTTTAACGCAAATCTACAATACCGATGCAAACGGTAATAAGATGTTAAAAAGCTGAC P L T Q I Y N T D A N G N K I V K K A D	6600
6601	GGAAAATGGTATGAACTGAATGCTGATGGTACGGCGAGTAACAAAGAAGTGACACTTGGT G K W Y E L N A D G T A S N K E V T L G	6660
6661	AACGTGGATGCAAACGGTAAGAAAAGTTGTGAAAGTAACCGAAAATGGTGGGATAAGTGG N V D A N G K K V V K V T E N G A D K W	6720
6721	TATTACACCAATGCTGACGGTGCTGCCGATAAAACCAAAAGGCGAAGTGAGCAATGATAAA Y Y T N A D G A A D K T K G E V S N D K	6780
6781	GTTTCTACCGATGAAAACACGTTGTCCGCCCTGATCCGAACAATCAATCGAACGGCAA V S T D E K H V V R L D P N N Q S N G K	6840

14 / 26

6841	GGCGTGGTCATTGACAATGTGGCTAATGGCGAAATTCTGCCACTTCCACCGATGCGATT	6900
	G V V I D N V A N G E I S A T S T D A I	
6901	AACGGAAGTCAGTTGTATGCCGTGGCAAAAGGGTAACAAACCTGCTGGACAAAGTGAAT	6960
	N G S Q L Y A V A K G V T N L A G Q V N	
6961	AATCTTGAGGGCAAAGTGAATAAAGTGGCAACGTGCAGATGCAGGTACAGCAAGTGCA	7020
	N L E G K V N K V G K R A D A G T A S A	
7021	TTAGCGGCTTCACAGTTACCAAGCCACTATGCCAGGTAAATCAATGGTTGCTATTGCG	7080
	L A A S Q L P Q A T M P G K S M V A I A	
7081	GGAAGTAGTTATCAAGGTCAAATGGTTAGCTATCGGGTATCAAGAATTCCGATAAT	7140
	G S S Y Q G Q N G L A I G V S R I S D N	
7141	GGCAAAGTGATTATTCGCTTGTCAAGGCACAACCAATAGTCAGGTAAAACAGGCGTTGCA	7200
	G K V I I R L S G T T N S Q G K T G V A	
7201	GCAGGTGTTGGTACCAAGTGGTAAAGTTGGATTATCTCTCT <u>AAAAAGCGGCATTTGCC</u>	7260
	A G V G Y Q W	
7261	<u>GCTTTTTTATGGGTGGCTATTATGTATCGT</u> 7291	

**FIG.\_3G**

15 / 26

**FIG.\_4**

16 / 26

**FIG.\_5A**

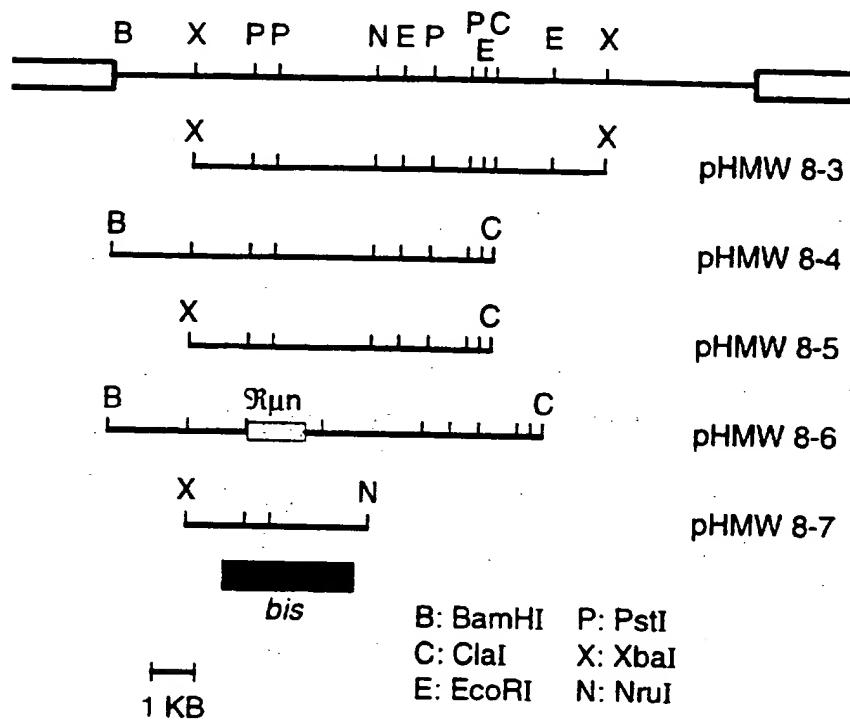
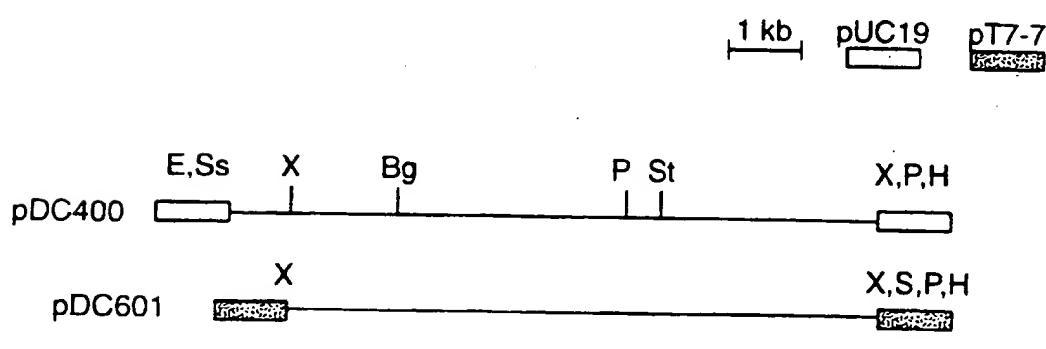
17 / 26

544 T...GNNGAKTEINKDGLTITPANGAGANNANTISVTKDGISAGGQSVKN 590  
541 VASGLRAYDDANFDVLNNSATDLNRHVEDAYKGLLNNEKNANKQ. PLVT 589  
|.|||: :|||||.||.||.:|.: :||||||| ||:||..:|| |:|. .  
591 VVSGLKKFGDANFDPLTSSADNLTKQNDAYKGLTNLDEKGTDKQTPVVA 640  
590 DSTAATVGDLRKLGWVVS 607  
|.||||||||| ||||:|  
641 DNTAATVGDLRGLGWVIS 658

**FIG.\_5B**

18 / 26

## Restriction maps of phage 11-17 and plasmid pT7-7 subclones

**FIG.\_6****FIG.\_7**

19 / 26

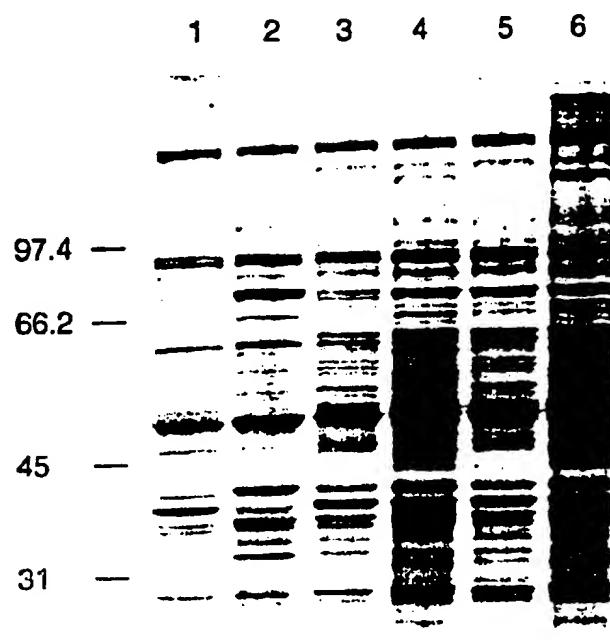


FIG.-8

1 2 3 4 5 6

1 2 3 4 5 6

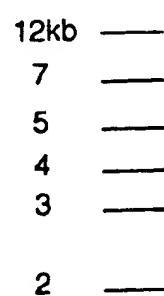
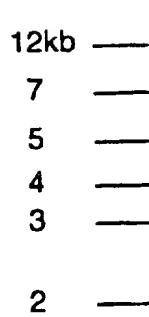
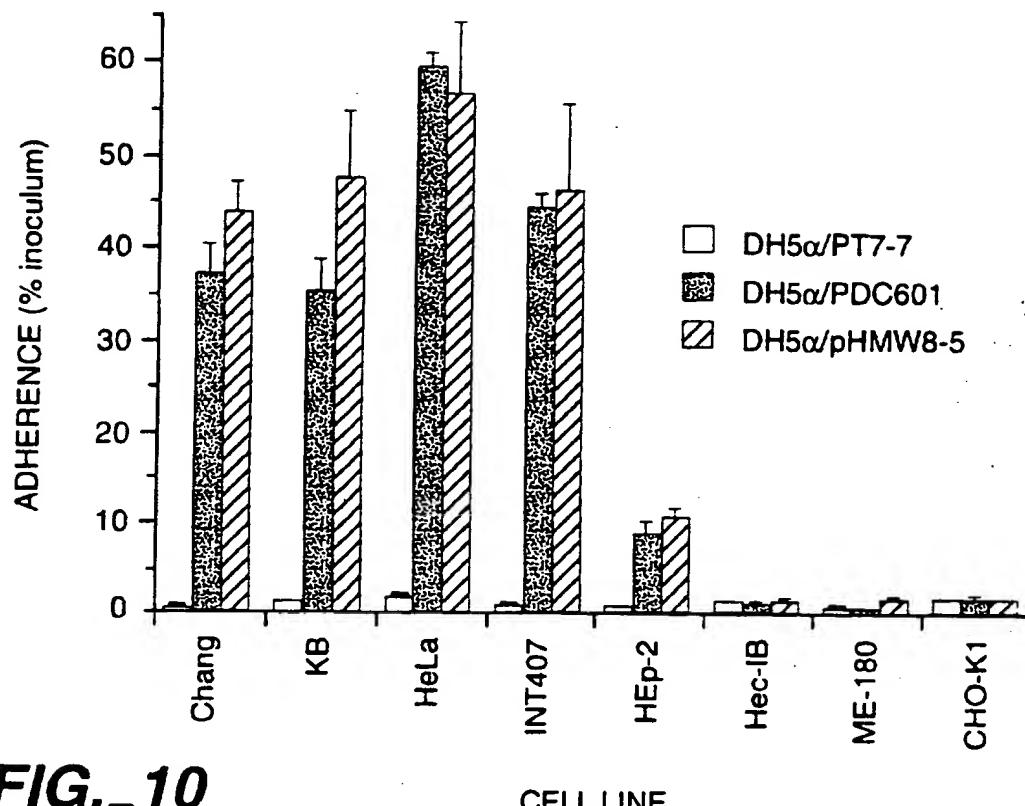


FIG.-9A

FIG.-9B

20 / 26

**FIG.\_10**

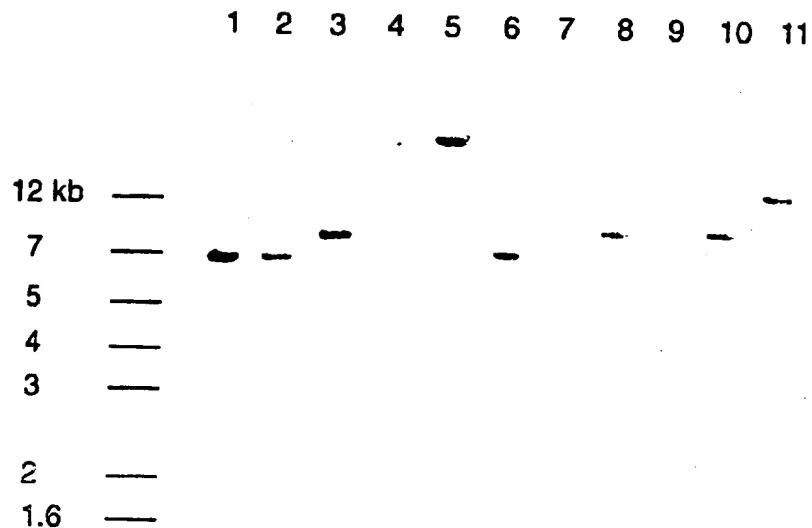
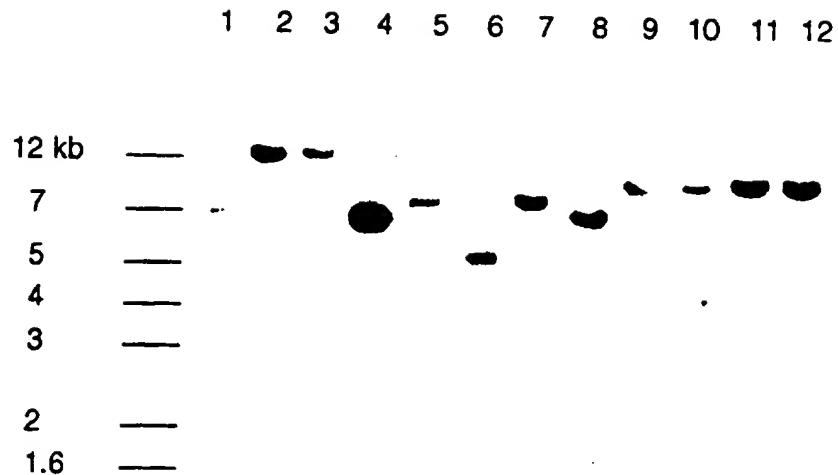
CELL LINE

1

HA2	MNKIFNVIWN	VMTQTWVVS	ELTR
HA1	MNKIFNVIWN	VVTQTWVVS	ELTR
HMW1	MNKIYRLKFS	KRLNALVAVS	ELAR
HMW2	MNKIYRLKFS	KRLNALVAVS	ELAR
AIDA-1	MNKAYSIIWS	HSRQAWIVAS	ELAR
Tsh	MNRIYSLRYS	AVARGFIAVS	EFAR
SepA	MNKIYYLKYC	HITKSLIAVS	ELAR
Consensus	MNKIY--IWS	-VTQ-W--VS	ELAR

**FIG.\_11**

21 / 26

**FIG.\_12****FIG.\_13**

1 ATGAACAAAA TTTTAAACGT TATTGGAAT GTTGTGACTC AAACTTGGGT  
51 TGTGTTATCT GAACTCACTC GCACCCACAC CAAATGCGCC TCCGCCACCG  
101 TGGCAGTTGC CGTATTGGCA ACCCTGTTGT CCGCAACGGT TCAGGCGAAT  
151 GCTACCGATG AAAACGAAGA TGATGAAGAA GAGTTAGAAC CCGTACAACG  
201 CTCTGTTTA AGGTGGAGCT TCAAATCCGC TAAGGAAGGC ACTGGAGAAC  
251 AAGAGGGAAC AACAGAGGTA ATAAATTGA ACACAGATTG ATCAGGAAAT  
301 GCAGTAGGAA GCAGCACAAAT CACCTTCAAA GCCGGCGACA ACCTGAAAAT  
351 CAAACAAAGC GGCAATGACT TCACCTACTC GCTGAAAAAA GAGCTGAAAA  
401 ACCTGACCAG TGTTGAAACT GAAAAATTAT CGTTTGGCGC AAACGGCAAT  
451 AAAGTTGATA TTACCAAGTGA TGCAAATGGC TTGAAATTGG CGAAAACAGG  
501 TAACGGAAAT GGTCAAAACA GTAATGTTCA CTTAACGGT ATTGCTTCGA  
551 CTTTGACCGA TACGCTTGCC GGTGGCACAA CAGGACACGT TGACACCAAAC  
601 ATTGATGCGG TTAATTATCA TCGCGCTGCA AGCGTACAAG ATGTGTTAAA  
651 CAGCGGTTGG AATATCCAAG GCAATGGAAA CAATGTCGAT TTTGTCCGTA  
701 CTTACGACAC CGTGGACTTT GTCAATGGCG CGAATGCCAA TGTGAGCGTT  
751 ACGGCTGATA CGGCTCACAA AAAGACAAC GTCCGTGTGG ATGTAACAGG  
801 CTTGCCGGTT CAATATGTTA CGGAAGACGG CAAAACCGTT GTGAAAGTGG  
851 GCAATGAGTA TTACAAAGCC AAAGATGACG GTTCGGCGGA TATGAATCAA  
901 AAAGTCGAAA ACGGCGAGCT GGCGAAAACC AAAGTGAAT TGGTATCGGC  
951 AAGCGGTACA AATCCGGTGA AAATTAGCAA TGTTGCAGAC GGCAACGGAAG

**FIG.\_14A**

1001 ACACCGATGC GGTCAGCTTT AAGCAATTAA AAGCCTTGCA AGACAAACAG  
1051 GTTACGTTGA GCACGAGCAA TGCTTATGCC AATGGCGGTA CAGATAACGA  
1101 CGGCAGGCAAG GCAACTCAAA CTTTAAGCAA TGGTTTGAAT TTTAAATTAA  
1151 AATCTAGCGA TGGCGAGTTG TTGAAAATTAA GCGCGACCAG CGATACGGTT  
1201 ACTTTTACGC CGAAAAAAAGG TTCCGGTACAG GTTGGCGATG ATGGCAAGGC  
1251 TTCAATTCA AAAGGTGCAA ATACAACGTGA AGGTTTGGTT GAGGCTTCTG  
1301 AATTGGTTGA AAGCCTGAAC AAACCTGGTT GGAAAGTAGG GGTTGAGAAA  
1351 GTCGGCAGCG GCGAGCTTGA TGGTACATCC AAGGAAACTT TAGTGAAGTC  
1401 GGGCGATAAA GTAACTTTGA AAGCCGGCGA CAATCTGAAG GTCAAACAAAG  
1451 AGGGCACAAA CTTCACTTAC GCGCTCAAAG ATGAATTGAC GGGCGTGAAG  
1501 AGCGTGGAGT TTAAAGACAC GGCGAATGGT GCAAACGGTG CAAGCACGAA  
1551 GATTACCAAA GACGGCTTGA CCATTACGCT GGCAAACGGT GCGAATGGTG  
1601 CGACGGTGAC TGATGCCGAC AAGATTAAAG TTGCTTCGGA CGGCATTAGC  
1651 GCGGGTAATA AAGCAGTTAA AAACGTCGCG GCAGGGCGAAA TTTCTGCCAC  
1701 TTCCACCGAT GCGATTAACG GAAGCCAGTT GTATGCCGTG GCAAAGGGG  
1751 TAACAAACCT TGCTGGACAA GTGAATAATC TTGAGGGCAA AGTGAATAAA  
1801 GTGGGCAAAC GTGCAGATGC AGGTACTGCA AGTGCATTAG CGGCTTCACA  
1851 GTTACCAACAA GCCACTATGC CAGGTAATC AATGGTTTCT ATTGGGGAA  
1901 GTAGTTATCA AGGTCAAAT GGTTAGCTA TCGGGGTATC AAGAATTCC  
1951 GATAATGGCA AAGTGATTAT TCGCTTGTCT GGCAACAACCA ATAGTCAAGG  
2001 TAAAACAGGC GTTGCAGCAG GTGTTGGTTA CCAGTGG

**FIG.\_14B**

1 MNKIFNVIWN VVTQTVVVVS ELTRTHTKCA SATVAVAVLA TLLSATVQAN  
51 ATDENEDDEE ELEPVQRSVL RWSFKSAKEG TGEQEGTTEV INLNTDSSGN  
101 AVGSSTITFK AGDNLKIKQS GNDFTYSLKK ELKNLTSVET EKLSFGANGN  
151 KVDITSDANG LKLAKTGNGN GQNSNVHLNG IASTLTDTLA GGTTGHVDTN  
201 IDAVNYHRAA SVQDVLSNGW NIQGNGNVND FVRTYDTVDF VNGANANVSV  
251 TADTAHKKTT VRVDVTGLPV QYVTEDGKTV VKVGNEYYYKA KDDGSADMNQ  
301 KVENGELAKT KVKLVSASGT NPVKISNVAD GTEDDAVSF KQLKALQDKQ  
351 VTLSTSAYA NGGTDNDGGK ATQTLSNGLN FFKFSSDGEL LKISATGDTV  
401 TFTPKKGSVQ VGDDGKASIS KGANTTEGLV EASELVESLN KLGWKGVEK  
451 VGSGELDGTS KETLVKSGDK VTLKAGDNLK VKQEGTNFTY ALKDELTGVK  
501 SVEFKDTANG ANGASTKITK DGLTITLANG ANGATVTDAD KIKVASDGIS  
551 AGNKAVKNVA AGEISATSTD AINGSQLYAV AKGVTNLAGQ VNNLEGKVNK  
601 VGKRADAGTA SALAASQLPQ ATMPGKSMVS IAGSSYQQQN GLAIGVSRIS  
651 DNGKVIIRLS GTTNSQGKTG VAAGVGYQW

**FIG.\_15**

25 / 26

1 MNKIFNVIWNVVTQTWVVVSELTRTHTKASATVAVAVLATLLSATVEAN 50  
 1 MNKIFNVIWNVVTQTWVVVSELTRTHTKASATVAVAVLATLLSATVQAN 50  
 51 NNTPVTNKLKAYGDANFNFTNNSIADAEKQVQEAYKGLNLNEKNASDKL 100  
 51 .....ATDENEDDEELEPVQRSLRWSFSAKEG. 80  
 101 LVEDNTAATVGNLRKLGWVLSSKNGTRNEKSQQVKHADEVLFEGKGGVQV 150  
 81 TGEQEGTTEVINL.....NTDSSGNAVGSSITFKAGDNLKI 117  
 151 TSTSENGKHTITFALAKDLGVKTATVSDTLTIGGAAAGATTPKVNVT 200  
 118 KQSGND....FTYSLKELKNLTSVETEKLSPGANGN.....KVDITS 156  
 201 TTDGLKFAKDAAGANGDTTVHLNGIGSTLTDLGVSPATHIDGGDQSTHY 250  
 157 DANGLKLAKTGNGNGQNSNVHLNGIASTLTDLAGGTTGHVDTNIDAVNY 206  
 251 TRAASIKDVLNAGWNIGVKAGSTTGQSENVDVHTYDTVEFLSADTETT 300  
 207 HRAASVQDVLSNGWNIQ.....GNGNVDFVRTYDTVDFVNGANANV 248  
 301 TVTVDSKENGKRTEVKIGAKTSVIKERDGKLFTGKANKETNKVDGANATE 350  
 249 SVTADTAHKTTVRVDVTGLPVQYVTEDGKTVVKVGNEYKAKDDGSADM 298  
 351 DADEGKGLVTAKDVIDAVNKTGWRIKTTDANGQNGDFATVA.....SG 393  
 299 NQKVENGELAKTKVKLVSASGTNPVKISNVADGTEDDAVSFKQLKALQD 348  
 394 TNVTFASGNGTTATVTNG.....TDGITVKYDAKVGDGKLDGDKI 434  
 349 KQVTLSTSNAVANGTDNDGGKATQTLSNGLNFKFKSSDGELLKISA... 395  
 435 AADTTALTVDG..KNANNPKGVADVASTDEKLVTAKGLVTALNSLW 482  
 396 TGDTVTFTPCKGSVQVGDDGKASISKGANTTE.GLVEASELVESLNKLGW 444  
 483 TTTAAEADGGTLGDNASEQEVKAGDKVTFKAGKNLKVQEGANFTYSLQD 532  
 445 KVGVEKGSGELDGTSETLVKGDKVTLKAGDNLKVQEGTNFTYALKD 494  
 533 ALTGLTSITL...GTGNNGAKTEINKGLTIT...PANGAGANNANTISV 576  
 495 ELTGVKSVEFKDTANGANGASTKITKDGLTITLANGANGATVTDADKIKV 544  
 577 TKDGISAGGQSVKNVSVGLKKFGDANFDPLTSSADNLTKQNDDAYKGLTN 626  
 545 ASDGISAGNKAVK..... 557

26 / 26

977 NVANGDISATSTDAINGSQLYAVAKGVTNLAGQVNLEGKVNKGKRADA 1026  
||||.||:|||||||||||||||||||||||||||||||||||||||||||||||||  
558 NVAAGEISATSTDAINGSQLYAVAKGVTNLAGQVNLEGKVNKGKRADA 607

1027 GTASALAASQLPQATMPGKSMVAIAGSSYQGQNGLAIGVSRISDNGKVII 107.6  
||||||||||||||||||||.|||||||||||||||||||||||||||||||||  
608 GTASALAASQLPQATMPGKSMVSIAGSSYQGQNGLAIGVSRISDNGKVII 657

1077 RLSGTTNSQGKTGVAAGVGYQW 1098  
|||||||||||||||||||||  
658 RLSGTTNSQGKTGVAAGVGYQW 679

**FIG.\_16B**

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/04031

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 6 C12N15/31 C07K14/285 A61K39/102 C07K16/12 // (C12N15/31, C12R1:21)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 6 C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 10936 (MICROCARB INC) 9 July 1992 see claims 10-15,25-36 ---	1,6, 13-16,19
X	WO,A,94 00149 (MICROCARB INC ;KRIVAN HOWARD C (US); SAMUELS JAMES E (US); NORBERG) 6 January 1994 see claims 5,7-23 ---	1-6, 13-16,19
		-/-
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		<input checked="" type="checkbox"/> Patent family members are listed in annex.
* Special categories of cited documents :		
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2 Date of the actual completion of the international search		Date of mailing of the international search report
19 August 1996		03.09.96
Name and mailing address of the ISA		Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl. Fax (+ 31-70) 340-3016		Gurdjian, D

**INTERNATIONAL SEARCH REPORT**

In. . . . . on patent family members

Internat	Application No
PCT/US	96/04031

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9210936	09-07-92	CA-A- 2098598 EP-A- 0565590 JP-T- 6508346 WO-A- 9400149	22-06-92 20-10-93 22-09-94 06-01-94
WO-A-9400149	06-01-94	CA-A- 2098598 EP-A- 0565590 JP-T- 6508346 WO-A- 9210936 CA-A- 2138765 EP-A- 0647139 JP-T- 7509693	22-06-92 20-10-93 22-09-94 09-07-92 06-01-94 12-04-95 26-10-95
WO-A-9602648	01-02-96	AU-B- 3097295	16-02-96

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/04031

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INFECT. IMMUN., 1992, vol. 60, no. 4, pages 1302-1313, XP000578343 BARENKAMP S J ET AL: "Cloning, expression, and DNA sequence analysis of genes encoding nontypeable <i>Haemophilus</i> <i>influenzae</i> high-molecular-weight surface-exposed proteins related to filamentous hemagglutinin of <i>Bordetella</i> <i>pertussis</i> " see the whole document ---	1,6. 13-16
X	INFECTION AND IMMUNITY, 62 (8). 1994. 3320-3328., XP000578342 BARENKAMP S J ET AL: "Genes encoding high-molecular-weight adhesion proteins of nontypeable <i>Haemophilus influenzae</i> are part of gene clusters" see the whole document ---	1,6. 13-16
X	105TH ANNUAL MEETING OF THE AMERICAN PEDIATRIC SOCIETY AND THE 64TH ANNUAL MEETING OF THE SOCIETY FOR PEDIATRIC RESEARCH, SAN DIEGO, CALIFORNIA, USA, MAY 7-11, 1995. PEDIATRIC RESEARCH, 37 (4 PART 2). 1994. 170A., XP000579256 BARENKAMP S J ET AL: "Identification of a second family of high molecular weight adhesion proteins expressed by nontypable <i>Haemophilus influenzae</i> (NTH1)" see abstract ---	1,6. 13-16
T	SCIENCE (WASHINGTON D C), 269 (5223). 1995. 496-498, 507-512., XP002010838 FLEISCHMANN R D ET AL: "Whole-genome random sequencing and assembly of <i>Haemophilus influenzae</i> Rd" see example ADHESIN ---	1-12
P,X	MOLECULAR MICROBIOLOGY, 19 (6). 1996. 1215-1223., XP000579265 BARENKAMP S J ET AL: "Identification of a second family of high-molecular-weight adhesion proteins expressed by non-typable <i>Haemophilus influenzae</i> " see the whole document ---	1,2,6,7. 11,13-15
P,X	WO,A,96 02648 (AMERICAN CYANAMID CO ;BACTEX INC (US); GREEN BRUCE A (US); BRINTON) 1 February 1996 see claims 10-12 -----	1,6. 13-16
2		

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US96/04031

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 20 - 21 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2.  Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.  Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest** The additional search fees were accompanied by the applicant's protest No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

In. citation on patent family members

Internat. Application No  
PCT/US 96/04031

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9210936	09-07-92	CA-A- 2098598 EP-A- 0565590 JP-T- 6508346 WO-A- 9400149		22-06-92 20-10-93 22-09-94 06-01-94
WO-A-9400149	06-01-94	CA-A- 2098598 EP-A- 0565590 JP-T- 6508346 WO-A- 9210936 CA-A- 2138765 EP-A- 0647139 JP-T- 7509693		22-06-92 20-10-93 22-09-94 09-07-92 06-01-94 12-04-95 26-10-95
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